Final Analysis
Statistical Analysis Plan (SAP)

Protocol Title:	A multi-center, randomized, open-label, non-inferiority trial to evaluate the efficacy and safety of a single, oral dose of zoliflodacin compared to a combination of a single intramuscular dose of ceftriaxone and a single oral dose of azithromycin in the treatment of patients with uncomplicated gonorrhoea
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		3.3 Trial treatments and assessments	Table 2 randomization schedule clarified in line with protocol update v5.0/12-Jul-2023
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V3.0 / 14-Sep-2023	Third version	6.2	Clarified definition of no pre-existing resistance.
		7.1.2	Add indeterminate culture outcome to definition of microbiological response for clarity.
		7.2.2	AE date imputation removed since AE date not allowed to be partial.
		8.1	Specify exclusion of data at site 710-005 from summary tables and figures
		8.4.2	Definition of HIV positive status
		8.4.3	Add separate summary of STI from sexual history questionnaire.
		8.6.4	Definition on Late Eradication Rate in the context of exploratory objectives
		8.7.2	Definition of eGFR added.

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LIST OF ABBREVIATIONS

The following abbreviations will be used within this SAP.

Abbreviation or special term	Explanation
AE	Adverse Event
ALT	Alanine transaminase
ART	Anti-retroviral therapy
AST	Antimicrobial susceptibility test
ATC	Anatomical Therapeutic Chemical
AUC	Area under the curve
BMI	Body Mass Index
CI	Confidence Interval
CIOMS	Council for International Organizations for Medical Science
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
Clinical-ITT	Clinical intention to treat
CTR	Clinical Trial Report
CT	Chlamydia trachomatis
CTCAE	Common Terminology Criteria for Adverse Events
DSMB	Data and safety monitoring board
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
eGFR	Estimated glomerular filtration rate
FBC	Full Blood Count
FDA	Food Drug Administration
GPHS	Government and Public Health Solutions
HIV	Human immunodeficiency virus
HLGT	Higher Level Group Term
ICH	International Conference on Harmonisation
IM	Intramuscular
IMP	Investigational Medicinal Product
KG	Kilogram
LFT	Liver function tests

LR	Logistics Regression
MAR	Missing at random
MedDRA	Medical dictionary for regulatory activities
MI	Multiple Imputation
MIC	Minimum inhibitory concentration
Micro-ITT	Microbiological intention to treat
Modified micro-ITT	Modified –microbiological intention to treat
MNAR	Missing not at random
MOP	Manual of procedures
MSM	Men who have sex with Men
NAAT	Nucleic acid amplification test
NG	Neisseria gonorrhoeae
NI	Non-inferiority
PK	Pharmacokinetics
PMM	Pattern Mixture Model
PO	per os
PP	Per Protocol
PrEP	Pre-exposure prophylaxis
PT	Preferred Term
PV	Pharmacovigilance
SAE	Serious Adverse Event
SAF	Safety Population
SAP	Statistical Analysis Plan
SD	Standard Deviation
SOC	System Organ Class
STI	Sexually transmitted infections
SUSAR	Suspected unexpected serious adverse reaction
TEAE	Treatment Emergent Adverse Event
TFLs	Tables, Figures and Listings
TOC	Test of Cure
USA	United States of America
WGS	Whole genome sequencing
WHO	World Health Organization

1 INTRODUCTION

The purpose of this Statistical Analysis Plan (SAP) is to provide detailed descriptions of the statistical methods, data derivations and data displays for trial protocol STI_Zoli001 Version 5.0 "A multi-center, randomized, open-label, non-inferiority trial to evaluate the efficacy and safety of a single, oral dose of zoliflodacin compared to a combination of a single intramuscular dose of ceftriaxone and a single oral dose of azithromycin in the treatment of patients with uncomplicated gonorrhoea" dated 12 July 2023 for final analysis. The table of contents and templates for the tables, figures, and listings (TFLs) will be produced in a separate document.

Any deviations from this SAP will be described and justified in the Clinical Trial Report (CTR).

The preparation of this SAP has been based on International Conference on Harmonization (ICH) E9 guideline (6).

All data analyses and generation of TFLs will be performed using SAS 9.4® or higher.

2 TRIAL OBJECTIVES

2.1 Primary objective(s)

The primary objective of this trial is to assess the efficacy of a single, oral, 3 grams (g) dose of zoliflodacin compared to a combination of a single intramuscular (IM) injection of 500 milligram (mg) dose of ceftriaxone and a single 1 g oral dose of azithromycin for the treatment of uncomplicated urogenital gonorrhoea

2.2 Secondary objective(s)

2.2.1 Safety Objective:

- To assess the safety and tolerability of a single, oral, 3 g dose of zoliflodacin compared to a combination of a single IM 500 mg dose of ceftriaxone and a single 1 g oral dose of azithromycin in participants with uncomplicated gonorrhea

2.2.2 Efficacy Objectives:

- To determine microbiological cure rate of pharyngeal gonorrhoea after administration of a single, oral, 3 g dose of zoliflodacin compared to a combination of a single IM 500 mg dose of ceftriaxone and a single 1 g oral dose of azithromycin
- To determine microbiological cure rate of rectal gonorrhoea after administration of a single, oral, 3 g dose of zoliflodacin compared to a combination of a single IM 500 mg dose of ceftriaxone and a single 1 g oral dose of azithromycin
- To determine the clinical cure rate of symptomatic gonorrhoea in male participants after administration of a single, oral, 3 g dose of zoliflodacin compared to a combination of a single IM 500 mg dose of ceftriaxone and a single 1 g oral dose of azithromycin
- To determine the microbiological cure rate of urogenital gonorrhoea among women and men respectively, after administration of a single, oral, 3 g dose of zoliflodacin compared to a combination of a single IM 500 mg dose of ceftriaxone and a single 1 g oral dose of azithromycin
- To determine the microbiological cure rate of *Neisseria gonorrhoeae* (NG) at urethral or cervical sites in the subset of participants with pre-existing resistance to antibiotics commonly used for NG treatment (including to ceftriaxone alone, to azithromycin alone, and to both)
- To determine the antimicrobial susceptibility profile of gonococcal strains isolated from participants with uncomplicated gonorrhoea at baseline and the Test of cure (TOC) visit
- To determine the eradication rate of NG nucleic acid from urethral, cervical, rectal, pharyngeal specimens after administration of a single, oral, 3 g dose of zoliflodacin compared to a combination of a single IM 500 mg dose of ceftriaxone and a single 1 g oral

dose of azithromycin

2.2.3 Pharmacokinetics (PK) objective (sub-trial only):

To evaluate the plasma PK profile after a single, oral, 3 g dose of zoliflodacin:

- in adult participants (≥ 18 years old) with uncomplicated gonorrhoea who are HIV negative or HIV positive
- in HIV negative adolescent participants (≥ 12 and < 18 years old) with uncomplicated gonorrhoea

2.3 Exploratory objective(s)

- To determine the late eradication rate of NG nucleic acid from urethral, cervical, rectal, pharyngeal specimens after administration of a single, oral, 3 g dose of zoliflodacin compared to a combination of a single IM 500 mg dose of ceftriaxone and a single 1 g oral dose of azithromycin
- To determine the late relapse/re-infection rate of NG from urethral, cervical, rectal, pharyngeal specimens after administration of a single, oral, 3 g dose of zoliflodacin compared to a combination of a single IM 500 mg dose of ceftriaxone and a single 1 g oral dose of azithromycin
- To investigate relationships between pre-treatment and post-treatment NG isolates within those participants whose cultures at TOC (day 6 ±2) and EOT (day 30 ±3) are found positive for NG and identify possible determinants of zoliflodacin resistance

3 TRIAL DESIGN

3.1 General trial design

This trial is a Phase III, interventional, multi-center, randomized controlled, open label, double-dummy, non-inferiority trial evaluating the safety and efficacy of a 3 g oral dose of zoliflodacin compared to a combination of a single IM 500 mg dose of ceftriaxone and a single 1 g oral dose of azithromycin for the treatment of uncomplicated gonorrhoea.

Participants will present to the clinic for assessment of eligibility after informed consent is signed on Day 1 (-1 day). Should eligibility be confirmed, they will be randomised to either the zoliflodacin group or the ceftriaxone/azithromycin combination group, undergo baseline assessments and dosed with the trial treatment on the same or next day. Assessments for Visit 1 can be performed over 2 days as long as ALL Baseline assessments and Dosing are performed on the 2nd day. The day that is considered Day 1 is the day of Dosing.

Those participants who are assigned to the zoliflodacin group and who will also have consented to be enrolled in the PK sub-trial will undergo blood sampling on Day 1 and return to the clinic on Day 2 for further blood sampling.

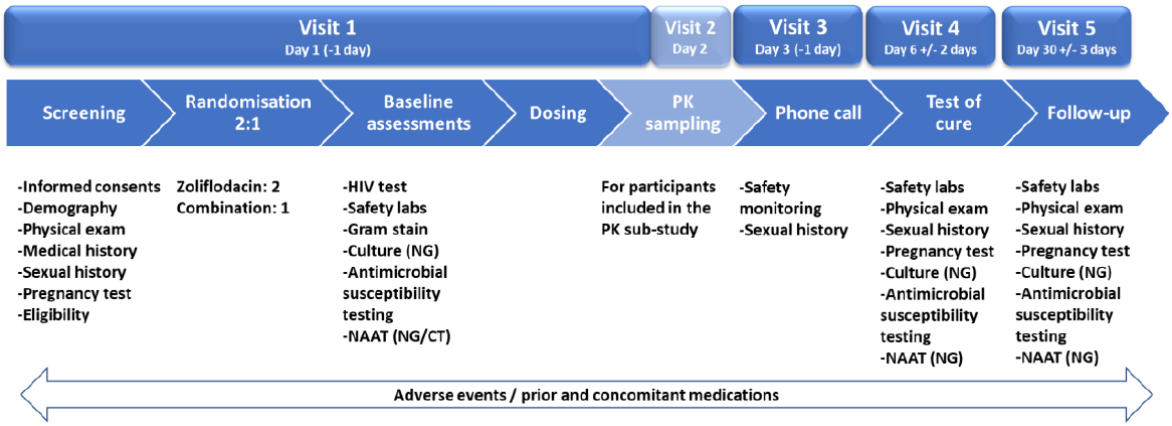
Sites staff will call participants on Day 3 for safety monitoring purposes and to enquire about sex behaviour since the previous clinic visit. Participants will return to the clinic on Day 6 for the TOC visit, involving safety and efficacy assessments (primary endpoint for the trial will be assessed at the TOC visit). Participants will be asked to return to the clinic for the EOT visit on Day 30 for final safety and efficacy assessments.

The trial consists of following visits and phone calls:

- Screening period/ Randomization (Visit 1, Day 1 [-1 day])
- Baseline assessments (Visit1, Day 1)
- Dosing (Visit 1, Day 1)
- PK Sampling (Visit 2, Day 2)
- Phone call (Visit 3, Day 3 [-1 day])
- Test of Cure (Visit 4, Day 6 [±2 days]))
- Follow-up (Visit 5, Day 30 [±3 days]))

For further details on the schedule of assessments refer to Table 2 below.

Figure 1: Trial design schematic



CT: *Chlamydia trachomatis*; HIV: human immunodeficiency virus; NAAT: nucleic acid amplification test; NG: *Neisseria gonorrhoeae*; PK: pharmacokinetics.

Note: Culture and NAAT assessments at visits 4 and 5 are conditional to results at previous visits as per the schedule of assessments (Table 2).

The target sample size is 696 evaluable participants: 464 in the zoliflodacin group and 232 in the comparator group including 20-30 adolescents ($\geqslant$12 and < 18 years old), with an estimated randomisation sample size of approximately 928. Accounting for anticipated screening failures , the maximum enrolment sample size is estimated to be around 1092.

The total duration for individual participants will be 30 days (± 3 days), unless the participant discontinues from the study or withdraws consent (refer to study protocol section 9 for details of reasons for study withdrawal). The trial treatment will be a single dose of either the investigational treatment or the comparator treatment. The duration of the follow-up period will be 29 days (± 3 days). The end of trial is defined as the last participant's last visit. Overall, recruitment into the trial is expected to last for approximately 3.5 years.

The trial is conducted in The Netherlands, Belgium, Thailand, South Africa and United States of America (USA).

3.2 Randomization and blinding

The randomization sequence will be obtained by computer-generated random numbers and provided to each trial site through a web-based randomization system (Flex Advantage). Trial participants will be assigned to receive either zoliflodacin or a ceftriaxone/azithromycin combination in a 2:1 allocation ratio. Unequal randomization has been chosen to increase the number of individuals from which safety data is gathered. Randomization will be performed using random permutated blocks of 3, 6, and 9 with stratification by sex at birth. At each trial site, a dedicated pharmacist or designated clinician will be responsible for monitoring drug storage and for dispensing trial drugs in accordance with the treatment allocation.

Blinding

This will be an open-label trial (with measures put in place to keep sponsor global staff blinded where feasible, described in a blinding convention). With a double-dummy design, participants would have to receive an IM injection and a tablet as well as an oral sachet, which would be likely to decrease their willingness to participate in the trial. Moreover, placebo injections might not be acceptable from an ethical perspective, and as a consequence the majority of gonorrhoea trials conducted over the past 30 years that included ceftriaxone treatment arm have been unblinded trials.

The laboratory staff who performs assessment of the primary outcome will be blinded to treatment allocation. This is achievable as the treatment allocation will not be mentioned on the trial specimen sent to the laboratory. Samples collected throughout the trial will be anonymized and a unique participant identifier will be used for sample tracking and result reporting. Laboratory results will be entered in the eCRF by site staff, or reported directly to data management as per pre-determined data specifications. For sites participating in the PK sub-trial, it will be ensured that trial personnel handling PK samples are different from the laboratory personnel handling microbiological samples.

With the exception of medical monitors and country specific personnel who interact regularly and are required to advise unblinded site teams, the sponsor remains blinded as detailed in the Blinding convention document. All the data will remain blinded to the sponsor until database lock, except in case of expedited reporting of suspected unexpected serious adverse reaction (SUSAR).

An unblinded statistician at ICON plc will be available should specific unblinded requests from the data safety monitoring board arise.

Procedures for unblinding

As this is an open-label trial, procedure for emergency unblinding is not applicable (in a medical emergency where knowledge of the blinded treatment is necessary for the treatment of an adverse event).

Unblinding of SUSARs for safety reporting is described in the Safety Management Plan.

3.3 Trial treatments and assessments

The participants in this trial will be randomized into either the below treatments on Day 1/ Visit 1:

- Trial treatment: zoliflodacin (3 g per os (PO)).
- Comparator treatment: ceftriaxone (500 mg) + azithromycin (1 g).

Administration of Trial Treatments:

Trial treatments will only be dispensed and administered to individuals enrolled in the trial and who have been randomized. Participants randomized to the zoliflodacin group will receive a single 3 g PO dose (one sachet) of zoliflodacin reconstituted with water. Detailed information on the preparation and administration of zoliflodacin can be found in the IMP preparation and administration instruction.

Administration of Comparator Trial Treatments:

Participants randomized to the comparator group will receive ceftriaxone as a 500 mg IM injection and azithromycin as four 250 mg oral tablets. Detailed information on the preparation and administration of zoliflodacin can be found in the IMP preparation and administration instruction.

Study Assessments:

A detailed description of procedures and assessments to be conducted during this trial is summarized in the Scheduled of Trial Assessments in Table 2 below.

Table 2: Schedule of Trial Assessments

Visit number	**1[1]**			**2**	**3**	**4**	**5**	
Timing (window)	**Day 1 (-1 day)**	**Day 1**		**Day2**	**Day 3 (-1 day)**	**Day 6 (± 2 days)[2]**	**Day 30 (± 3 days)**	
Visit type	**Screening**	**Baseline**	**Dosing**	**PK**	**Phone call**	**Test of cure (TOC)**	**End of trial**	**Early withdrawal / unscheduled visit[3]**
Screening assessments								
Informed consent[4]	X							
Demographics[5]	X							
Medical history	X							
Sexual history[6]	X				X	X	X	X
Targeted physical examination[7]	X[8]					X	X	X
Pregnancy test	X					X	X	X
Assessment of eligibility	X							
Randomization	X[17]							
Laboratory investigations								
HIV testing[9]		X						
Full blood count (FBC) and differentials		X				X	X	X
Liver function tests (LFTs)[10]		X				X	X	X
Creatinine and estimated glomerular filtration rate (eGFR)		X				X	X	X
Urethral/endocervical Gram stain and microscopy examination[11]		X						
Efficacy outcomes								
Urogenital NG culture and NAAT		X				X[12]	X[13]	
Rectal NG culture and NAAT		X				X[12]	X[13]	
Pharyngeal NG culture and NAAT		X				X[12]	X[13]	
AST[14]		X				X	X	
NAAT for CT (urogenital)		X						

NAAT for CT (rectal)		X						
NAAT for CT (pharyngeal)		X						
Pharmacokinetic sampling[15]		X		X				
Food intake[16]			X					
Trial treatment administration			X					
Adverse events	----------→							
Prior / concomitant medications	----------→							

AST: antimicrobial susceptibility testing; CT: *Chlamydia trachomatis;* HIV: human immunodeficiency virus; MG: *Mycoplasma genitalium;* NAAT: nucleic acid amplification test; NG: *Neisseria gonorrhoeae.*PK: pharmacokinetics.

1. Assessments for Visit 1 can be performed over 2 days as long as ALL Baseline assessments and Dosing are performed on the 2nd day. Day 1 is the day of dosing. For details see CSP Section 5.2.1.
2. In case ANY of the NG microbiological test results for a specific anatomical site is not available prior to the collection of the TOC visit specimens, both NG culture and NAAT microbiology specimens should be collected for that particular anatomical site and microbiological evaluations should be performed.
3. 3. If a participant has completed scheduled visits as per protocol and returns to the clinic on an additional occurrence on a day that does not correspond to a scheduled trial visit, site staff should handle them as per their routine practice. In this case no assessment is mandated as per protocol. See CSP Section 5.3 for details.
4. Including counselling on sexual risk behaviour, and partner(s) notification and treatment
5. Including year of birth, gender, sex at birth, ethnicity
6. See Appendix 2 in trial protocol for sexual history questionnaires
7. Including clinical assessment of gonorrhoea
8. Screening physical examination will act as baseline too
9. HIV testing is mandatory only for participants to the PK sub-study. For all other participants, HIV testing is proposed, but not mandatory for inclusion in the study. An HIV test result obtained on the day of enrolment may be used as baseline assessment. Participants with a documented positive HIV diagnosis or documented prescription of ART also do not need to be tested again.
10. Alanine transaminase (ALT) and total and direct bilirubin (direct bilirubin only required if total bilirubin is elevated)
11. A Gram stain result obtained on the day of enrolment may be used as baseline assessment
12. In cases where both baseline NG NAAT and NG culture results are negative, no further samples should be taken at subsequent visits. This applies to each anatomical site respectively.
13. Only take sample if:
    - symptoms at any anatomical site are present. If symptoms at any anatomical site are observed, take samples from all 3 anatomical sites.
    - a negative culture and a positive NG NAAT results were obtained at TOC. This applies to each anatomical site respectively..
14. Only for culture samples with confirmed identification of *N. gonorrhoea*
15. Only for participants who consent to participate in the PK sub-study. The PK sub-study will take place in select sites only. See CSP Section 7.3 for time points.
16. Food must be taken before trial treatment. Details on timing of food intake in relation with dosing time and required food content are available in the MOP
17. For participants of the PK sub-study only, randomisation can occur on Day -1 (Screening) once eligibility has been confirmed and participants can return to complete baseline assessments and receive study treatment the following calendar day, to assist with scheduling of the visit.

4 TRIAL ENDPOINTS

4.1 Primary Efficacy Endpoint

- Microbiological cure as determined by culture at urethral or cervical sites at TOC (day 6 ±2)

4.2 Secondary Endpoint(s)

The secondary endpoints of this trial are:

4.2.1 Safety Endpoint:

- Incidence, severity, causality, and seriousness of treatment-emergent adverse events and the evaluation of changes from baseline in safety laboratory test results and physical examinations

4.2.2 Efficacy Endpoints:

- Proportion of participants with microbiological cure as determined by culture at pharyngeal sites at TOC (day 6 ±2)
- Proportion of participants with microbiological cure as determined by culture at rectal sites at TOC (day 6 ±2)
- Proportion of male participants with clinical cure at TOC (day 6 ±2)
- Proportion of female and male participants respectively with microbiological cure as determined by culture at cervical or urethral site at TOC (day 6 ±2)
- Proportion of participants with microbiological cure as determined by culture at urethral or cervical sites at the TOC visit and for whom the baseline antimicrobial susceptibility profile indicated pre-existing resistance to antibiotics commonly used for NG treatment (including to ceftriaxone alone, to azithromycin alone and to both)
- Antimicrobial susceptibility profile of gonococcal strains isolated at baseline and at TOC (day 6 ±2)
- Proportion of participants with a negative NG nucleic acid amplification test (NAAT) from urethral or cervical sites at TOC (day 6 ±2)
- Proportion of participants with a negative NG NAAT from oropharyngeal sites at TOC (day 6 ±2)
- Proportion of participants with a negative NG NAAT from rectal sites at TOC (day 6 ±2)

4.2.3 PK Sub-trial endpoints

- Plasma PK parameters of zoliflodacin in HIV negative and HIV positive adult participants
- Plasma PK parameters of zoliflodacin in HIV negative adolescents (≥ 12 and < 18 years old)

4.3 Exploratory endpoint(s)

- Out of participants with a positive NG NAAT test and a negative NG culture at TOC for

specified anatomical site, the below will be determined:

- Proportion of participants with a negative NG NAAT and culture from urethral or cervical sites at the end of the trial (day 30 +/- 3 days)
- Proportion of participants with a negative NG NAAT and culture from oropharyngeal sites at the end of the trial (day 30 +/- 3 days)
- Proportion of participants with a negative NG NAAT and culture from rectal sites at the end of the trial (day 30 +/- 3 days)

- Out of participants with symptoms at end of the trial (day 30 +/- 3 days), the following will be determined:
  - Proportion of participants with positive NAAT and/or culture at each anatomical site respectively at end of the trial (day 30 +/- 3 days)
  - Antimicrobial susceptibility profile of gonococcal strains isolated at the end of the trial (day 30 +/- 3 days)
- Whole genome sequencing (WGS) for strain typing and assessment of resistance determinants of NG isolates from the TOC and end of trial visits plus all baseline isolates (urogenital, pharyngeal and rectal) from the same participants with possible microbiological failure

5 SAMPLE SIZE AND POWER

The efficacy of the combination therapy 500 mg IM ceftriaxone and 1 g oral azithromycin has been used as the reference in the SOLITAIRE-U trial. This trial has assessed the non-inferiority of a single dose of 1 g oral solithromycin in comparison with the combination therapy in uncomplicated urogenital gonorrhoea; it reported 84.5% (95% CI, 72.4 to 87.1%) microbiological cure rate under the combination therapy (14). Initially, a 10% non-inferiority (NI) margin for the treatment difference in proportion of ceftriaxone/azithromycin combination and zoliflodacin was selected, based on the US Food and Drug Administration (FDA) guidelines on drug development for NG (13), and the initial assumption that there would be no treatment difference between the comparator (parenteral ceftriaxone and oral azithromycin) and zoliflodacin. An unequal allocation ratio has been chosen to provide sufficient data on the safety of zoliflodacin (2).

During the study however, GARDP assessed the cure rates of all recent Phase 2 and 3 trials investigating the efficacy of a new oral investigational product for uncomplicated gonorrhoea (1, 4, 10,11). This indicated that when comparing an oral therapy (even with 100% susceptibility) against a parenteral combination that rarely fails, a true underlying treatment difference of at least -4% should be assumed, rather than the assumption of equal underlying cure rates used in the initial sample size calculation. In addition, Mitrani-Gold et al. published in 2022 a systematic literature review and meta-analysis (using a weighted, non-iterative random-effects model) to estimate the microbiological response rate and 95% CIs for ceftriaxone and proxy-for-placebo (14). The analysis proposed that by reviewing data from more recent studies, a NI margin of 15% was shown to robustly preserve estimated ceftriaxone treatment effect in a Phase 3 microbiologically evaluable population. Using the same methodology, GARDP conducted a conservative analysis in a microbiologically evaluable population comparing ceftriaxone treatment effect in the micro-ITT population with the proxy-for-placebo estimate, which showed a margin of 12% would preserve 57% of the estimated ceftriaxone treatment effect. At a Type C interaction held in October 2022, the US FDA indicated that a NI margin of 12% could be acceptable.

Consequently, it will be considered that a single oral 3 g of zoliflodacin will be non-inferior to a combination of a single IM 500 mg dose of ceftriaxone and a single 1 g oral dose of azithromycin if the upper bound of the 2-sided 95% CI for the microbiological cure rate of the combination therapy minus zoliflodacin would be less than 12% (prespecified NI margin for the primary endpoint).

A blinded pooled data analysis of sample sizing assumptions was also performed. Considering the expected -4% treatment difference and a NI margin of 12%, it was calculated that 696 participants evaluable for the micro-ITT analysis set (464 in the intervention group and 232 in the control group, with a two-sided α of 5% and 2:1 allocation ratio) would provide approximately 90% power to show

that a single oral 3 g dose of zoliflodacin is non-inferior to a combination of a single IM 500 mg dose of ceftriaxone and a single 1 g oral dose of azithromycin with respect to microbiological cure rate at TOC.

Taking into account criteria for inclusion in the micro-ITT population (see Section 6.2) and blinded assessment of study conduct, it is estimated conservatively that a maximum of 25% of the randomized population would be potentially ineligible for inclusion in the micro-ITT population. Therefore, up to approximately 928 participants are to be randomized into the trial in order to achieve the target sample size of 696 in the micro-ITT population. Taking into account anticipated screening failures, the enrolment sample size remains estimated to be around 1,092.

6 ANALYSIS POPULATIONS

For each microbiological outcome and anatomical site (see Section 2 objectives and Section 4 endpoints), there will be three analysis population types used for the efficacy analysis: the micro-ITT, the evaluable and the PP population. The micro-ITT will be the primary analysis population while the evaluable and PP population will be the secondary. For clinical outcomes, the clinical cure and PP populations will be used.

6.1 Randomized Population

The Randomized population will include all participants who are randomized.

6.2 Micro-ITT population

The micro-ITT population will be defined separately for urogenital, rectal or pharyngeal body site populations and will include all randomized participants who had a positive NG culture from the relevant anatomical site at baseline and whose baseline AST result showed no pre-existing resistance to both ceftriaxone and azithromycin (i.e. participants with ceftriaxone MIC>0.25 µg/mL AND azithromycin MIC>1 µg/mL with be excluded from the population). It will include participants who do not comply with trial treatment, those who are lost-to-follow-up, those who are nonassessable for microbiological outcome, or who receive antibacterial therapy with activity against NG prior to the TOC visit. A participant's eligibility for the micro-ITT population will depend on the particular anatomical site in question.

Resistance to ceftriaxone and azithromycin will be defined as per CLSI [8] standards.

The micro-ITT population will be derived by anatomical site at baseline. For example, participants who receive the trial treatment and are found to have a negative NG culture at the urethral or cervical site at baseline (visit 1) will be excluded from the micro-ITT population and thus of the primary efficacy endpoint analysis and secondary analyses corresponding to the urethral or cervical sites. Participants who receive trial product and are found to be NG culture negative at the rectal or pharyngeal site at visit 1 will be excluded from the micro-ITT population for the corresponding secondary efficacy endpoint analyses at the rectal or pharyngeal sites.

The micro-ITT population will be used for the evaluation of the efficacy assessments.

6.3 Modified micro-ITT Population

The modified micro-ITT population will be defined separately for urogenital, rectal or pharyngeal body site populations and will include all randomized participants who had a positive NG culture from the relevant anatomical site at baseline (regardless of pre-existing resistance).

6.4 Clinical Cure Population

For the efficacy analyses of clinical cure rate, the clinical cure population will include all male at birth participants included in the micro-ITT population and who had at least one sign or symptom of urethral gonorrhoea at baseline.

6.5 Safety (SAF) Population

The safety population will include all randomized participants who have received any part of trial treatment.

6.6 PK population

The PK population will include all participants randomized to the zoliflodacin group who consent to participate to the PK sub-trial and from whom at least one valid PK sample post treatment is obtained.

6.7 Evaluable population

The evaluable population will be defined separately for urogenital, rectal or pharyngeal body site populations and will include all randomized participants who have a positive NG culture at baseline and whose baseline antimicrobial susceptibility testing result showed no pre-existing resistance to both ceftriaxone and azithromycin, who do not vomit within 30 mins of administration of zoliflodacin or azithromycin and who have a NG culture result at the TOC visit.

6.8 Per-Protocol (PP) population

The PP population will be defined separately for urogenital, rectal or pharyngeal body site populations and will include all participants in the micro-ITT population who meet all inclusion/exclusion criteria, complied with trial treatment, did not vomit within 30 mins of administration of zoliflodacin or azithromycin, did not receive any systemic antibiotic with known activity against NG prior to TOC visit, did not receive any of the prohibited medications, abstained from sexual intercourse or used condoms for vaginal, anal and oral sex prior to TOC and returned to the trial site for the TOC visit within the specified window (visit 4, trial day 6±2).

6.9 Clinical-PP population

The clinical-PP population will include male at birth participants in the PP population with at least one sign or symptom of gonorrhoea at baseline and with an evaluable clinical outcome:

6.10 Protocol deviations/violations and exclusions from analysis sets

All violations and exclusions of participants from analysis populations will be identified at the Classification Meeting just prior to trial unblinding, through clinical review input provided by Sponsor, using the following sources of information:

- Supportive participant listings, provided by the ICON lead statistician ahead of the Classification Meeting, based on data recorded on the eCRF.

- Protocol Deviation Logs, provided by ICON.

Further, deviations from protocol will be classified as important or non-important.

The important protocol deviations to be identified and to be included in the body of the CSR are:

- Failure to obtain informed consent
- Failure to meet Inclusion/Exclusion Criteria for randomized subjects
- Non-compliance with trial treatment
- Non-compliance with study assessment
- Prohibited medications used up to TOC
- Failure to conduct TOC in specified visit window
- Failure to abstain from sexual intercourse or use condoms up to TOC

7 STATISTICAL CONSIDERATIONS AND ANALYSIS

7.1 Derived Variables

The below table provide the list of derived variables for demographic and baseline characteristics, baseline derivations and other important derivations of variables applicable for this trial.

7.1.1 Demographics

Variables	Formula
Demographic and Baseline characteristics	
Age (years)	Age as collected in eCRF will be used.
Body mass index (BMI) (kg/m^2)	weight (kg)/[height (m)]^2
Derivation of Duration	
Trial day at any visit	Date of interest – date of first dose of trial drug. One day is added if this difference is ≥ 0
Baseline Derivations	
Baseline	The baseline value is defined as the last observation prior to or on the date of the trial drug administration. i.e. Day 1/Dose 1 Value.
Change from baseline	Post baseline value – Baseline value

7.1.2 Efficacy

Microbiological cure:

Microbiological cure is defined as a participant with a positive NG culture at baseline and a negative or indeterminate NG culture at the TOC visit for the specified body site. A positive NG culture is defined as a culture with confirmed microbiological identification by the central laboratory. An indeterminate culture at TOC is defined as a culture overgrown with normal flora.

Microbiological failure:

Microbiological failure is defined as a participant with a positive NG culture at baseline and at the TOC visit. A participant with a positive NG culture at baseline and who is nonassessable for microbiological outcome at TOC (e.g. who has a missing microbiological outcome at the TOC due to a missed visit or a lost/damaged microbiological specimen) is also considered a failure in the analysis in the micro-ITT population.

Clinical cure:

Clinical cure is defined as a male at birth participant experiencing resolution of ALL signs and symptoms of urogenital gonococcal infection that were present at enrolment, and no new symptoms.

Lost-to-follow-up:

For the purpose of the analysis, Lost-to-follow-up is defined as a participant who has missed his/her appointment after completing treatment and cannot be traced until and including the end of the expected follow-up period (30 days± 2 days).

Non-evaluable for microbiological outcome:

Non-evaluable for microbiological outcome is defined as a participant who did not have a urogenital positive culture result for NG at baseline (visit 1).

7.2 Handling of missing data and outliers

Prior to analysis, any missing data will be investigated and reasons for missing data obtained and summarized where possible. All attempts will be made to avoid any missing outcome data and to correct for it. In the primary analysis the definition of the micro-ITT includes participants who are non-assessable for microbiological outcome or who are lost-to-follow-up as microbiological failures. A sensitivity analysis will be performed in which we will replace missing outcomes using multiple imputation (MI).

MI method will be used for sensitivity analysis for primary endpoint to explore departures from assumptions about missing data. Otherwise, no imputation will be employed for the descriptive summaries and listings.

7.2.1 Missing data analysis methods for sensitivity analysis

The following sensitivity analyses of the primary endpoint will be performed using pattern mixture model (PMM) to determine the effect on the inference allowing data to be

- missing at random (MAR)
- missing not at random (MNAR)

Multiple Imputation (MI) using PMM:

In this approach, multiple imputed datasets will be generated based on the assumption of a monotone missing data pattern. The imputation will be performed using the monotone regression model to replace the missing values of the primary endpoint.

Details are provided in Section 8.6.2.1.

7.2.2 Handling of missing or incomplete dates

Imputation rules for missing or partial medication start/stop dates are defined below:

Missing or partial medication start date:

- If only DAY is missing, use the first day of the month.
- If DAY and Month are both missing, use the first day of the year.
- If DAY, Month and Year are all missing, use date the patient was enrolled.

Missing or partial medication stop date:

- If only DAY is missing, use the last day of the month.
- If DAY and Month are both missing, use the last day of the year.
- If DAY, Month and year are all missing, assign ‘continuing’ status to stop date

8 STATISTICAL METHODS

8.1 General statistical conventions

All statistical procedures will be completed using SAS version 9.4 or higher.

Unless otherwise stated, all statistical testing will be two-sided and will be performed using a significance (alpha) level of 0.05. Two-sided 95% confidence intervals (CI) will be provided when relevant. For the primary end point, statistical testing of the NI will be performed at significance level of 0.05 with a 12% NI margin in difference of proportions.

For each microbiological outcome and anatomical site (Section 4 Trial Endpoints), there will be three analysis population types used for the efficacy analysis: the micro-ITT, evaluable and the PP population. The micro-ITT population will be the primary analysis population while the evaluable and PP population will be the secondary. For clinical outcomes, the clinical cure and PP populations will be used.

For all microbiological assessments determining analysis population and efficacy outcome, central laboratory results will be used.

Continuous variables will be summarized using descriptive statistics, including number of participants (n), mean, standard deviation (SD), median, minimum and maximum. One additional decimal point for mean and median and two additional decimal points for SD will be used in addition to the number of decimal points used for the measured values.

For categorical variables, summaries will include counts of participants and percentages. Percentages will be rounded to one decimal place.

For statistical analyses “baseline” refers to the last observation before treatment, i.e., pre-treatment values measured on the treatment day (baseline visit, day 1), and if missing or not evaluated, values will not be imputed. A HIV test result obtained post-dose but on the day of enrolment will be considered as baseline if no pre-treatment value exists.

All summaries will be presented by treatment group, unless otherwise specified. Analyses will be performed by visit, irrespective of any time window deviations.

All participant data, including those derived, will be presented in individual participant data listings. Unless otherwise stated, unscheduled visit results will be included in date/time chronological order, within participant listings only. All listings will be sorted by investigational site, participant number, date/time and visit. The treatment group (Test, Reference) as well as participant’s sex and age will be stated on each listing. Unless otherwise stated, data listings will be based on the Participant Randomized Population.

Data obtained from participants enrolled at site 710-005 will not be included in any summary tables or figures, and will be included in listings only. This is due to data integrity findings during GARDP

site audit indicating a serious breach of GCP.

8.2 Participant disposition

Participant disposition information will be summarized by treatment group and overall. The number and percent of participants who are randomized, who took a dose of trial drug, who were randomized and not treated, who complete the trial and who withdraw early from the trial will be presented.

The primary reason for early withdrawal will also be tabulated.

The number of participants randomized will be used as the denominator for the percentage calculation. Participant disposition will be listed. In addition, a listing will be provided to include the unmet inclusion/exclusion criteria at Screening/Baseline Visits.

The number and percent of participants in each analysis population will also be tabulated.

Screen failures will also be listed with reason for not being randomized.

Treatment Misallocations:

If a participant was:

- Randomized but not treated, then they will be reported under their randomized treatment group for efficacy analyses. However, they are by definition excluded from the safety analyses as actual treatment is missing.
- Randomized but took incorrect treatment, then they will be reported under their randomized treatment group for all efficacy analysis, but will be reported under the treatment they actually received for all safety analyses.

8.3 Protocol deviations

The number of participants excluded from Randomized, Micro-ITT (urogenital), Micro-ITT (pharyngeal), Micro-ITT (rectal), modified Micro-ITT (urogenital), modified Micro-ITT (pharyngeal), modified Micro-ITT (rectal), Clinical-cure, SAF, PK, Evaluable (urogenital), Evaluable (pharyngeal), Evaluable (rectal), PP and Clinical-PP populations and reasons for exclusion will be summarized by treatment group and overall for all screened patients.

Population membership details will be listed, including reason for exclusion from each population .

All important protocol deviations identified will be summarized by treatment group and overall.

A listing will be provided for protocol deviations identified based on data recorded on the eCRF and/or protocol deviation Logs from ICON Medical (on Randomized participants).

8.4 Demographics and baseline characteristics

8.4.1 Demographics

Age, height, weight, body mass index (BMI), sex at birth, gender, race and ethnicity at baseline will be summarized using descriptive statistics as described in Section 8.1 by treatment group and overall in the Micro-ITT (urogenital), Micro-ITT (pharyngeal), Micro-ITT (rectal) and Randomized

Populations.

8.4.2 Baseline and disease characteristics

The categorical baseline characteristics such as previous or coexisting sexually transmitted infections, urine pregnancy test, Human Immunodeficiency virus (HIV) testing and sexual history at baseline will be summarized by treatment group for the Micro-ITT (urogenital), Micro-ITT (pharyngeal), Micro-ITT (rectal) and Randomized Population.

There will be additional table which will be summarizing baseline characteristics of participants by treatment groups with no missing outcome will be presented to assess whether these characteristics were balanced between the treatment groups when only participants with non-missing outcomes are considered.

HIV positive status will include participants who have a positive HIV test at baseline (a HIV test result obtained post-dose but on the day of enrolment will be considered as baseline if no pre-treatment value exists), medical history of HIV, or who are receiving anti-retroviral indicated for HIV treatment.

Gonorrhoea clinical assessment at baseline for males at birth will be summarized in the Clinical-cure and Micro-ITT (urogenital) population. Gonorrhoea clinical assessment at baseline for females at birth will be summarized in the Micro-ITT (urogenital) population.

An individual listing will be provided for demographic and baseline characteristics.

Presumptive (local lab) evaluations of *Neisseria gonorrhoeae* (NG) culture, NG-Nucleic Acid Amplification Testing (NAAT), *Chlamydia trachomatis* NAAT and *Mycoplasma genitalium* NAAT at urogenital, rectal and pharyngeal sites will be summarized.

Organisms identified by the central lab at baseline will be summarized by body site (although *Neisseria gonorrhoeae* cultures will be isolated and sent to central lab, contaminants or mis-identification may be possible). Results of all cultures by the local and central labs at baseline and TOC will be listed.

8.4.3 Medical / Surgical history

A summary of medical history will be presented by system organ class (SOC) and preferred term (PT) using Medical Dictionary for Regulatory Affairs® (MedDRA) Version 23.0 or higher. The table will be based on the SAF population.

The details of medical history findings will be listed by participants and sites.

A summary of STI history (from the sexual history questionnaire) will be presented by SOC and PT similarly. The table will be based on the SAF population.

8.4.4 Prior and concomitant medications

Medications used in this trial will be coded by using the latest available version of the World Health

Organization (WHO) Drug Dictionary Standard or Enhanced and categorized as follows:

Prior medications: are defined as those medications with a start date/time prior to dose of trial drug.

Concomitant medications: are defined as those medications with a start date/time on or after dose of trial drug, or with start date/time prior to dose and stop date/time after dose of trial drug. Therefore, a medication may be considered both prior and concomitant.

Prior medications and concomitant medications will be summarized using frequency tables by anatomical therapeutic chemical (ATC) class level 1, ATC level 2 and preferred name by treatment group on the SAF Population.

Details for imputing missing or partial start and/or stop dates of medication are described in Section 7.2.2.

In addition, prior medications and concomitant medications will be listed with ATC classification and preferred term by participants and sites.

8.5 Extent of exposure

8.5.1 Treatment duration

Since the participants will receive only one dose of trial medication, treatment duration will not be calculated.

An individual listing will be provided to include details of drug administration by participant and sites.

8.5.2 Treatment compliance

All efforts will be taken to achieve 100% of treatment compliance at each Investigator site by site staff.

Compliance information will not be summarized.

8.6 Efficacy analyses

8.6.1 Analysis methods

The primary analysis of efficacy will be based on the primary endpoint which is defined as a negative culture to NG at urethral or cervical sites at TOC (day 6 ±2) among participants included in the micro-ITT population.

For the primary endpoint analysis, the proportion of participants with negative culture to NG at urogenital sites at TOC will be presented by treatment group. The point estimate and 2-sided 95% CI with Newcombe score method (9) for the treatment difference in proportion of ceftriaxone/azithromycin combination and zoliflodacin will be presented for the primary endpoint. Newcombe 95% CIs for the treatment difference in proportion of ceftriaxone/azithromycin combination and zoliflodacin will be calculated from SAS Proc Freq, option Riskdiff for treatment

difference with treatment groups based on randomized treatment. The exact 95% CIs (Clopper-Pearson method) will be used for the proportion of participants with negative culture within a treatment group.

NI will be concluded if the upper limit of the 95% CI of the treatment difference of ceftriaxone+ azithromycin combination minus zoliflodacin is less than the NI margin of 0.12 (or 12%) for the primary efficacy endpoint.

Subgroup analyses will be described in Section 8.6.3.1

8.6.1.1 Multiplicity

Multiplicity adjustment will not be done for the primary endpoint because there is only one. Statistical testing of secondary endpoints will not adjust alpha for multiplicity, and results should be interpreted with caution.

8.6.1.2 Treatment by center interaction analysis (multi-center trial)

There will not be any treatment-by-center interaction analysis performed for the endpoints.

8.6.2 Analysis of primary efficacy endpoints

The primary objective of this trial is to assess the efficacy of zoliflodacin compared to ceftriaxone/azithromycin combination in Microbiological cure as determined by culture at urethral or cervical sites at TOC (day 6 ±2).

The null and alternative hypotheses are expressed as:

$$H_0: P_2–P_1 \geqslant 12\% \text{ versus } H_a: P_2–P_1 < 12\%$$

where P_2 and P_1 denote the proportions of negative culture to NG at urethral or cervical sites at TOC (day 6 ±2) in ceftriaxone/azithromycin combination and zoliflodacin group respectively.

The primary endpoint will be summarized with individual proportions and its 95% CI using Clopper-Pearson method within a treatment. And, the difference in the proportions of participants with negative culture to NG at urethral or cervical sites at the TOC visit will be calculated using Newcombe method and its 2-sided 95% CIs will be presented in percentages (6).

The primary efficacy analysis will claim zoliflodacin as non-inferior to the ceftriaxone/azithromycin combination if the upper bound of the 2-sided 95% CI for the microbiological cure rate of the combination therapy minus zoliflodacin is less than the 12% prespecified NI margin.

The primary efficacy analysis will be carried out using the participants from the Micro-ITT (urogenital) population, which includes participants who are non-assessable for microbiological outcome or who are lost-to-follow-up. This efficacy analysis will also be performed on the Modified Micro-ITT (urogenital), Evaluable (urogenital) and PP population as part of the secondary objectives.

A similar analysis of microbiological cure rate at TOC at pharyngeal sites in relevant pharyngeal

populations, and of microbiological cure rate at TOC in rectal sites in relevant rectal populations, will also be performed.

8.6.2.1 Sensitivity Analyses for primary efficacy endpoint

The primary analysis will be repeated in the Evaluable (urogenital) population.

After protocol amendment 3, samples were collected for TOC at an out of window assessment. A sensitivity analysis of the primary analysis will be performed including assessment of TOC samples collected out of window.

In addition, to include all randomized participants and replace missing values of microbiological cure at TOC, MI methods will be implemented as a sensitivity analysis.

To take into account the possibility that missing outcomes are not completely at random and to trial the departures from assumptions about missing data at random, PMM will be used for both MAR and MNAR mechanisms, and the robustness will be tested using a tipping point analysis.

Multiple imputed datasets will be generated based on the assumption of a monotone missing data pattern. The missing data will be imputed with predicted values from a multivariable logistic regression model that will include these observed participant data and that will be adjusted for baseline covariates (*e.g.* trial centre, age, history of unprotected sex). The multivariable model will yield a probability of a Bernoulli process that is used to generate values for the outcome data. The probability will be used to impute the missing outcome value by rounding it to the nearest integer (0 or 1) or by sampling from a Bernoulli distribution. To avoid any bias, the imprecision due to imputation will be accounted for by means of multiple imputation (M=20 imputations) and seed value 1,234. Hence, multiple datasets with imputed outcomes will be generated and results will be pooled by using standard techniques taking into account the variation between imputed data sets (5). To take into account the possibility that missing outcomes are not completely at random, we will also fit a pattern mixture model under MAR that will be expanded to allow for the context of MNAR (5).

General stepwise procedure for MI:

- Step i) Imputation – Multiple copies of the original dataset will be generated by replacing missing values at TOC (Day 6) using Logistic Regression (LR) model (assuming a monotone pattern of missing data). Imputed microbiological cure will be used for the analysis.
  Based on the resulting logistic regression equation, a probability is generated for each case with missing data and a Bernoulli draw is made for that probability, producing imputed values of 0 or 1.

- Step ii): Analysis – Risk difference for proportions at TOC (Day 6) will be performed for each of the multiple imputed datasets to estimate the relevant standard errors and difference in proportions on imputed dataset.

- Step iii): Pooling – The results from the multiple imputed datasets will be combined based on Rubin's rules for pooling mean and variance to produce a unique point estimate (Risk Difference) and confidence intervals.

Results of sensitivity analyses will be presented graphically by an estimation of the treatment effect (microbiological cure rate) in the combination therapy group, in the zoliflodacin group and in both groups depending on the departures from assumptions about missing data.

The efficacy analysis mentioned above will also be performed on the PP population as part of the secondary objectives.

8.6.3 Analysis of secondary efficacy endpoints

The secondary efficacy endpoints will be analyzed for the comparison of treatment arms.

For the comparisons of

- The proportions of microbiological cure at TOC between both treatment groups at pharyngeal sites (in Micro-ITT (pharyngeal), Modified Micro-ITT (pharyngeal), Evaluable (pharyngeal) and PP populations)
- The proportions of microbiological cure at TOC between both treatment groups at rectal sites (in Micro-ITT (rectal), Modified Micro-ITT (rectal), Evaluable (rectal) and PP populations)
- The proportions of male at birth participants with clinical cure at TOC between both treatment groups (in Clinical Cure population and Clinical-PP populations).

Two-sided 95% CIs for the proportions and the difference of proportions will be calculated, where appropriate (using same methods as for primary endpoint).

In addition, the following comparison will be made using descriptive statistics only

- The proportions of participants with a negative NG NAAT from urethral or cervical sites at TOC between both treatment groups
- The proportions of participants with a negative NG NAAT from oropharyngeal sites at TOC between both treatment groups
- The proportions of participants with a negative NG NAAT from rectal sites at TOC between both treatment groups

These analyses will be conducted among participants that have a positive NAAT from the corresponding site at baseline.

To analyse the antimicrobial susceptibility testing (AST) profile, MIC breakpoint (per CLSI criteria and EUCAST [3] criteria) for antibiotics other than zoliflodacin will be applied. In addition the median, 90th percentile, 50th percentile and range of MICs will be reported for each antibiotic in the

panel by treatment group and site of infection at baseline and TOC. The proportion of isolates at or above MIC breakpoint (n/N) at baseline and TOC (if applicable) will also be reported by treatment group and by site of infection. Moreover, changes in MIC (changes of ≥2 doubling dilutions) between the baseline and TOC isolates will be reported for all participants with microbiological failure without any statistical comparison.

The proportion of participants with microbiological cure at TOC will be presented by body site (in micro-ITT analysis set by body site), by MIC (for zoliflodacin, azithromycin and ceftriaxone) at baseline and treatment group.

8.6.3.1 Subgroup Analyses

To determine consistency of effect, the primary endpoint will be analyzed for subgroups listed below using the same analysis as the primary outcome. Data will be presented in a table and a forest plot.

- Sex at birth (male vs female) (per eCRF)
- Gender (male vs female)
- Age (<18 vs 18-64 vs ⩾65)
- Body mass (⩽35–⩽50kg vs >50–⩽75kg vs >75–⩽100kg vs >100kg)
- HIV status (negative vs positive vs not collected)
- Race (Black vs White vs Asian vs Other)
- Region (EU vs South Africa vs Asia vs US)
- Fasted status (fed prior to dosing vs fasted (last meal >2 hours) prior to dosing)
- pre-existing resistance status (per CLSI urogenital result) to antibiotics commonly used for NG treatment (resistant to ceftriaxone alone vs resistant to azithromycin alone vs resistant to both vs susceptible to both)
- Received PrEP medication for HIV at baseline (Yes vs No)
- Risk factor MSM (Yes vs No)
- Sex worker (Never vs Sometimes vs Often)

Other baseline variables may also be included as subgroups, for example if a baseline imbalance is observed between treatment arms.

The forest plots will be inspected, and the presence of qualitative interactions (a situation where the treatment effect is above the NI margin for one subgroup level, and below the NI margin for the complement of the subgroup) may be assessed by a global interaction test.

Subgroup analysis may be repeated for key secondary endpoints.

8.6.4 Analysis of exploratory endpoints

All the exploratory analyses will be based on the micro-ITT by body site, unless otherwise stated. The exploratory endpoints will be analyzed descriptively, and will not use statistical testing.

The analysis of the below endpoint will be conducted among participants that have a positive NAAT from the corresponding site at baseline.

Late eradication of NG NAAT is defined as both a negative NG NAAT test and negative NG culture at end of trial visit.

The analysis of the below endpoints will be conducted out of participants with a positive NG NAAT test and a negative NG culture at TOC

- Late eradication rate from urethral or cervical sites at the end of the trial (day 30 +/- 3 days)
- Late eradication rate from oropharyngeal sites at the end of the trial (day 30 +/- 3 days)
- Late eradication rate from rectal sites at the end of the trial (day 30 +/- 3 days)

The analysis of the below endpoints will be conducted out of participants with symptoms at end of the trial (day 30 +/- 3 days):

- Rate of positive NG NAAT test and/or positive NG culture at each anatomical site respectively at end of the trial (day 30 +/- 3 days)
- Antimicrobial susceptibility profile testing (AST) of gonococcal strains isolated at the end of the trial (day 30 +/- 3 days) among those with a positive culture

For the AST, the following exploratory, descriptive comparisons will be summarized:

- AST profile (MIC distribution) of baseline and TOC isolates for participants with multiple sites of infection vs participants with unique site of infection.
- AST profile (MIC distribution) of baseline and TOC isolates for isolates that are resistant to fluoroquinolones at baseline vs isolates that are fluoroquinolone-sensitive.
- AST profile (MIC distribution) of baseline and TOC isolates for isolates that are resistant to other antibiotics commonly used for NG treatment (e.g. azithromycin and ceftriaxone, tetracyclines) at baseline vs isolates that are sensitive to those antibiotics.
- AST profile (MIC distribution) of baseline isolates of participants with subsequent microbiological failure vs microbiological cure (in the micro-ITT and evaluable populations.

The below exploratory endpoint will be descriptively analyzed in a separate report to the CSR.

- WGS of NG isolates from the TOC and end of trial visits plus all baseline isolates (urogenital, pharyngeal and rectal) from the same participants with possible microbiological failure

WGS will be conducted on all NG isolates cultured at the TOC and end of trial visits, as well as on pre-treatment isolates from the respective participants. The objective of the analysis is to investigate relationships between pre-treatment and post-treatment NG isolates within those participants whose cultures at TOC or end of trial are found positive for NG, evaluate the likelihood of re-infection vs treatment failure and identify possible genetic determinants of zoliflodacin resistance. Percentages and frequencies will be presented at TOC and end of trial visit.

8.7 Safety analyses

All the safety analyses will be conducted on the safety population (treated participants).

All definitions relative to safety endpoints are detailed in the following Sections.

All safety data will be summarized by treatment group.

The safety analyses of changes from baseline to a specific time point in safety variables (e.g., laboratory parameters, weight) will only include participants from the Safety population who have data available for both the baseline and the time point under consideration unless otherwise specified.

No statistical test will be performed.

For the continuous safety measures, both actual and change values will be summarized using descriptive statistics at baseline and scheduled visit.

8.7.1 Adverse events

All Adverse events (AEs) will be classified by Primary System Organ Class (SOC), higher level group term (HLGT) and Preferred Term (PT) according to the Medical Dictionary for Regulatory Activities (MedDRA) Version 23.0 or higher.

Adverse events will be sorted alphabetically by SOC and PT. In summaries by PT, AEs will be sorted by decreasing frequency according to the total column.

An AE will be considered as treatment emergent adverse events (TEAE) if it occurs or worsens on or after receipt of the trial drug. Details for imputing missing or partial start dates of adverse events are described in Section 7.2.2. Imputed Adverse Event dates will be used for determining treatment-emergence.

The occurrence of AEs and SAEs will be summarized in terms of incidence, as well as in terms of total number of AEs.

An AE overall summary table will be presented by treatment group and overall with the following information:

- All AEs
- All TEAEs
- Related TEAE
- Serious AEs (SAEs)
- Related SAEs
- TEAEs Leading to treatment discontinuation
- TEAEs by maximum severity (Mild, Moderate and Severe)
- TEAEs by maximum severity (CTCAE Grades)

The incidence of TEAEs will be summarized to include only one occurrence of a preferred term (PT) per participant per treatment. If a participant reports multiple occurrences of the same PT and treatment, then that preferred term will only be counted once. As with the PT, if a participant reports multiple TEAEs within the same system organ class (SOC) and treatment, that SOC will be counted only once.

When reporting adverse events by severity, in addition to providing a summary table based on the event selection criteria detailed above, summary table will also be provided based on the most severe event during the treatment period - independent of relationship to trial treatment.

All TEAEs, all TEAES by maximum severity, all TEAEs by maximum relationship, TEAEs leading to treatment discontinuation and all SAEs will be summarized by SOC, PT and treatment group using frequency counts and percentages (i.e., number and percentage of participants with an event) within treatment groups.

All AEs will be listed, but only treatment-emergent AEs (TEAEs) will be summarized by SOC and PT. A summary of SAEs, related SAEs and a summary of non-serious AEs will be presented by SOC and PT by treatment group. In addition, all SAEs will be listed by participant.

A summary of all AEs leading to death will be presented by SOC and PT by treatment group.

A summary of TEAEs by SOC, PT, and severity will be presented by treatment group. Severity will be graded by the investigator according to the Common Terminology Criteria for Adverse Events (CTCAE) as “Mild”, “Moderate”, “Severe”, “life-threatening” and “death”. If a participant reports multiple occurrences of the same PT/HLGT or SOC, only the most severe will be presented.

8.7.2 Clinical laboratory evaluations

For the purposes of summarization in both the tables and listings, all laboratory values will be presented in SI units. If a lab value is reported using a nonnumeric qualifier e.g., less than (<) a certain value, the given numeric value subtracting (1E-dp) will be used in the summary statistics, where dp is the number of decimal places shown in result e.g. if result = "< 0.05" then AVAL = 0.05 - (1E-2) = 0.04. Similarly if result is reported using greater than (>) then convert the number in result to numeric and add (1E-dp), where dp is the number of decimal places shown in result e.g. if result = "> 0.05" then AVAL = 0.05 + (1E-2) = 0.06.

Descriptive statistics (mean, standard deviation, median, interquartile range, minimum and maximum) will be used to summarize clinical laboratory data at baseline and during follow-up visits. Changes in laboratory results pre- and post-treatment will be examined. Shift tables showing the number of participants with a clinically significant value at baseline as well as at all post baseline visits will be presented per parameter.

Continuous baseline laboratory variables such as Alanine Aminotransferase (ALT) will be summarized by descriptive statistics in the same way as continuous demographic variables for the Micro-ITT, Clinical-cure and SAF Populations.

A summary table and box plot by visit and race will be presented in the SAF population for white cell count and absolute neutrophil count. Abbreviated patient profiles of participants with neutrophil count <1.0 x 10^9/L post-baseline will be presented, including demographic information, medical history, STI history, medications, AEs and key laboratory parameters such as neutrophils, platelets, red blood cells and white blood cells.

A table and figure of maximum post-dose ALT vs maximum post-dose total bilirubin, expressed as multiples of ULN, will be presented.

To ensure consistency in the eGFR values presented, eGFR will calculated programmatically from laboratory data for presentation in summary tables. For calculation of eGFR, the following formula (CKD-EPI) will be used (15):

$$eGFR = 142 \times min(Scr/\kappa, 1)^{\alpha} \times max(Scr/\kappa, 1)^{-1.200} \times 0.9938^{Age} \times 1.012[if\ female]$$

Where *Scr* is serum creatinine (mg/dL), κ is 0.7 for females and 0.9 for males, α is -0.241 for females and -0.302 for males, min indicates the minimum of Scr/κ or 1, and max indicates the maximum of Scr/κ or 1.

Both calculated eGFR and eGRF as reported by the investigator will be listed.

8.7.3 Physical examinations

Descriptive statistics for vital sign measurements (weight, BMI) and their changes from baseline, when appropriate, at each post-baseline visit will be presented by treatment group and overall. Height measurements collected at Day 1/Visit 1 will be carried forward for future visits to calculate BMI.

Vital signs data will be listed.

All other physical examination data (General appearance, Head, Eyes, Ears, Nose, Throat (HEENT), Neck, Lymph nodes, Cardiovascular/Heart, Pulmonary/Chest, Abdomen, Genitourinary, Extremities, Skin, Musculoskeletal and Neurological) will be summarized at each visit with percentages and frequencies along with abnormalities by treatment group and overall.

All physical examination data and abnormalities will be listed (including pre-treatment and post-treatment results). Shift tables from baseline to Visit 5/Day 30 of normal vs. abnormal assessments will be provided by treatment group.

8.7.4 Electrocardiograms (ECG)

ECG data are not collected for this trial.

8.7.5 Pregnancy

Positive pregnancy tests after dosing will be listed.

8.8 Other analysis

8.8.1 Pharmacokinetics (PK) analysis

The plasma concentration of zoliflodacin will be retained in SDTM dataset only.

The analysis of PK parameters will be addressed in a separate report.

8.9 Interim analysis

No formal interim statistical analysis is planned.

8.9.1 Data Safety Monitoring Board (DSMB)

An independent data and safety monitoring board (DSMB), composed of 3 members independent of the investigators and sponsor, will be constituted prior to trial implementation. Its role will be to monitor the accumulated trial data for participants' safety. Depending on this evaluation, the DSMB will make recommendations to the sponsor concerning the continuation, modification or termination of the trial. There will be no planned interim analysis in this trial.

The DSMB will consist of at least one STI clinical expert. Support from an independent statistician will be provided by ICON GPHS. An unblinded statistician will be available should specific requests requiring an unblinded statistician from the data safety monitoring board arise. Prior to trial start, a

DSMB charter and SOP will be established and the schedule for subsequent, interim meetings will be determined. The format of DSMB reports will also be discussed.

During the trial, the sponsor will be responsible for timely communicating to the DSMB all SAEs. In addition, the sponsor will communicate quarterly progress reports to the DSMB that will include safety listings and Council for International Organizations for Medical Science (CIOMS) reports.

The DSMB will be responsible for interpreting the information communicated by the sponsor and for determining:

- whether the overall incidence, seriousness and severity of AEs jeopardizes participants' safety
- whether the investigational drug poses significant, new, safety concern compared to the reference information.

9 CHANGES TO PLANNED ANALYSIS FROM TRIAL PROTOCOL

Clarification that the primary efficacy analysis will claim zoliflodacin as non-inferior to the ceftriaxone/azithromycin combination if the upper bound of the 2-sided 95% CI for the microbiological cure rate of the combination therapy minus zoliflodacin is less than (not less than or equal to) the 12% prespecified NI margin. This is in line with FDA guidance "Non-Inferiority Clinical Trials to Establish Effectiveness" (12).

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16.

GARDP_STI_Zoli001_SAP Final_v3.0

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