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

Clinical Trial Protocol

ClinicalTrials.gov identifier: NCT03959527

Short title	Zoliflodacin in uncomplicated gonorrhoea
Name of products	Zoliflodacin, ceftriaxone and azithromycin
Drug Class	Antibiotic
Phase	Phase III
Indication	Uncomplicated gonorrhoea
Trial Protocol Number	STI_Zoli001
EudraCT	2019-000990-22
IND number	139,105
Sponsor	GARDP, Chemin Camille-Vidart, 15, 1202 Geneva, Switzerland; Phone: +41 22 555 19 90
Protocol chair	Prof Edward Hook, University of Alabama at Birmingham, United States of America
Clinical Trial Protocol Version / Date	Version 5.0 of 12 July 2023
Clinical Trial Protocol Template Version/Date	Version 1.2 of 2 June 2014
Protocol Amendment Number / Date	Amendment 4 / 12 July 2023

CLINICAL TRIAL PROTOCOL SIGNATURE PAGE

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INVESTIGATOR SIGNATURE PAGE

I have read this protocol and agree that it contains all necessary details for carrying out this trial. I will conduct the trial as outlined herein and in compliance with GCP/GCLP, the principles outlined in the Declaration of Helsinki, all applicable regulatory requirements and local regulations. I will complete the trial within the time designated.

I will provide copies of the protocol and all pertinent information to all individuals responsible to me who assist in the conduct of this trial. I will discuss this material with them to ensure they are fully informed regarding the investigational product and the conduct of the trial.

I will use only the informed consent form approved by the sponsor or its representative and will fulfill all responsibilities for submitting pertinent information to the Institutional Review Board/Independent Ethics Committee (IRB/IEC) responsible for this trial if required by national law.

I agree that the sponsor or its representatives shall have access to any source documents from which case report form information may have been generated.

Principal investigator at each trial site

Investigator signature _____ _____

Date of Signature (DD/mmm/YY)

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Title
Institution
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PROTOCOL REVISIONS

Version / Date	Changes	Sections impacted
V 1.0 / 8 April 2019	Original version	
V2.0 / 9 August 2019	Addition of methylene blue test as possible NG diagnostic test in inclusion criterion #3	Summary, 4.1
	Clarification on barrier contraception required during participation in the study only	Summary, 4.1
	Update of exclusion criterion #22 as per SmPC of new source of ceftriaxone	Summary, 4.2
	Exclusion criterion #28 reworded into « previous randomisation in this clinical trial » to allow for re-screening	Summary, 4.2
	Clarification of treatment of participants diagnosed *a posteriori* as infected with CT at baseline	3.1
	Correction of stratification made on sex at birth	3.3
	Collection of full date of birth	5.1, 5.2.1
	Clarifications on clinical assessment of gonorrhoea	5.1, 5.2.1, 5.2.4, 5.2.5
	Removal of use of participant diary card	5.1, 5.2.1, 5.2.4, 5.2.5, 8.6
	Clarification of collection of microbiological samples	5.1, 5.2.1, 5.2.5, 7.4
	Collection of date/time of last meal taken prior to dosing for all participants	5.2.1
	Reference to the MOP for assessment of clinical cure	5.2.4
	Reference to pharmacy manual for study treatment storage and preparation	6.5, 6.6
	Clarification of physical examination of pharynx required	7.1
	Correction of total blood volume collected in accordance with ICF	7.2.1
	Clarification on collection of ART last dose for PK participants	7.3
	Correction of allowed PK sample processing time	7.3
	Infant follow up by paediatrician made optional	8.4
	Renaming of clinical ITT population into clinical cure population	11.2.2
	Removal of sexual behaviour questionnaire from protocol as provided to regulatory authorities and ethics committees as a standalone document for review	5.1, 5.2.1, 5.2.3, 5.2.4, 5.2.5, Appendices
	Correction of typographical errors and abbreviations	All
V3.0 / 22 November 2019	Head of Clinical Affairs (A. Janin) replaced by Study Medical Lead (M. Heep)	Protocol Signature Page
	Removal of CYP3A4 inhibitors group in PK sub-study	Summary,2 7.3
	Addition of justification for CYP3A4 exclusion	3.2.4
	Inclusion age of participants lowered to 12 years old	Summary, 2, 4.1, 7.3
	Clarification on methylene blue /gentian violet stain	Summary, 4.1
	Addition of exclusion criterion for CYP3A4 inhibitors	Summary,4.2
	Correction of unblinding procedure in case of SUSAR	3.4
	Clarification on HIV testing as per local practice	5.1, 5.2.1
	Clarification of capture of last food intake prior to dosing	5.1, 5.2.1
	Introduction of flexibility on timing of Visit 3	5.1, 5.2.3
	Correction of assessments to be performed at early withdrawal visits	5.1, 5.2.6
	Clarification of handling of unscheduled visits	5.3
	Update of total number of subjects exposed to zoliflodacin	6.1.2

Version / Date	Changes	Sections impacted
	Addition of forbidden concomitant CYP3A4 inhibitors	6.9
	Clarification on contacting medical monitor regarding newly diagnosed HIV positive participants	6.9
	Addition of nature of food intake around dosing time for PK participants	7.3
V4.0 / 22 October 2021	Correction of typos, minor edits and clarifications, updating of abbreviations	Throughout protocol
	Administrative changes: Updates of study team members and sponsor address, Advisory panel role clarification, Simplification of protocol signatures to follow to GARDP Standard Operating Procedure (SOP)	Clinical trial protocol signature page
	Contingency measure due to COVID-19 pandemic: Deletion of *Chlamydia trachomatis* (CT) assessment at Day 30 visit (exploratory endpoint)	Protocol summary, 2, 3.1, 5.1, 7.4, 11.4.2
	Contingency measure due to COVID-19 pandemic: Deletion of *Mycoplasma genitalium* (MG) assessment (exploratory endpoint)	Protocol summary, 2, 3.1, 5.1, 5.2.1, 7.4, 11.4.2
	Clarification of contraception requirements prior to inclusion in the trial (Inclusion criteria 5)	Protocol summary, 4.1, 8.4, Appendix 1
	Clarification of HIV testing requirements for study participation (Inclusion criteria 8)	Protocol summary, 4.1, 5.1, 5.2.1, 7.2.1
	Clarification of treatment of concomitant infections with systemic antibiotics with activity against *Neisseria gonorrhoeae* (NG) (Exclusion criteria 13)	Protocol summary, 4.2
	Clarification of selection of participants	Protocol summary, 4, 11.1
	Background information literature is updated to reflect most recent surveillance data on gonorrhoea infections	1.1
	Addition of recent *in vitro* data supporting the 3g dose and rewording of most common AE	1.2, 3.2.2
	Update of section 1.3 with latest information on gepotidacin clinical development and NABOGO trials preliminary results	1.3
	Update text to reflect the updated US Centers for Disease Control and Prevention (CDC) guidelines for treatment of uncomplicated gonorrhoea. References to national treatment guidelines are updated when applicable.	3.2.3
	Update of the clarification of CYP3A4 exclusion and mention of planned study STIZoli_003	
	Contingency measure due to COVID-19 pandemic: Addition of Section 3.2.5 Impact of COVID-19 pandemic on trial conduct and contingency measures in trial design justification	3.2.5
	Clarification of how randomisation is performed	3.3
	Clarification of section on blinding	3.4
	Update of overall trial duration	3.5
	Duration of Day 1 visit: Allowing Day 1 visit to happen over 2 days under specific conditions	5.1 and 5.2.1
	Addition of mandatory food intake before dosing	5.1 and 5.2.1

Version / Date	Changes	Sections impacted
V4.0 / 22 October 2021	Clarification of procedure to follow if microbiological assessments results are not back in time for the test of cure (TOC) visit	5.1, 5.2.4
	Addition of sexual history questionnaire assessment at early withdrawal visits	5.1, 5.2.6
	Clarification that direct bilirubin is only required to be performed when total bilirubin is out of normal range.	5.1, 7.2.1
	Addition of text to highlight that those participants who miss the TOC visit are considered treatment failure for primary endpoint analysis	5.2.4
	Contingency measure due to COVID-19 pandemic Clarification on handling of out-of-window visits and unscheduled visits	5.3
	Removal of summary of zoliflodacin non-clinical and clinical experience and referral to the Investigator Brochure (IB)	6.1.2
	Reference is added to the ceftriaxone and azithromycin United States prescribing information (USPI) in addition to the summaries of product characteristics (SmPC) as applicable.	6.2.1
	Contingency measure due to COVID-19 pandemic: Flexibilities for pharyngeal swab collection	7.2.2
	Contingency measure due to COVID-19 pandemic: NAAT testing allowed with other platforms than Hologic	7.2.2
	Clarification on food intake recommendations in the pharmacokinetic (PK) sub-study	7.3
	Clarification of section 8.1.1 adverse events (AE) 1. Assessment whether laboratory/procedures abnormalities should be considered as an AE 2. Clarification of AE definition 3. Clarification of special cases for AE occurring during screening period	8.1.1
	Minor clarification of the end of the AE reporting period	8.1.2
	Deletion of duplicated information and clarification of sponsor team in section 8.3.1 Reporting of SAEs to sponsor	8.3.1
	Clarification of events to be reported in an expedited manner	8.3.2
	Clarification of Exposure during pregnancies section	8.4
	Clarification of AE follow up	8.5
	Addition of new analysis population and additional sensitivity analysis	11.2, 11.2.3, 11.4.2
	Clarification of definition of microbiological failure	11.4.1
	Contingency measure due to COVID-19 pandemic: Addition of flexibility around monitoring visit format	13.1
	Addition of the trial clinicaltrials.gov number.	17.1
V5.0 / 12 July 2023	Administrative changes: Updates of study team members	Protocol Signature Page
	Increase of pre-specified non-inferiority margin to 12% for primary endpoint with rationale and re-estimation of evaluable patient population for microITT based on blinded pooled data analysis of study performance vs baseline assumptions	Summary, 3.2.1, 4, 11.1, 11.4.2

Version / Date	Changes	Sections impacted
V5.0 / 12 July 2023	Clarification that randomisation can occur on Day -1 and trial treatment administration the next calendar day (Day 1) to accommodate logistics of PK sampling (*Incorporation in protocol amendment of Note-to-file from 7 July 2022*)	5.1 (Table 3), 5.2.1
	Reference to UAB central laboratory deleted to reflect change of central lab vendor (*Incorporation in protocol amendment of Note-to-file from 30 January 2023*)	7.2.2, 11.7
	Correction of error around TOC results in Table 4 Assessments and use for clinical management; clarification of anatomical sites and indication for sampling at post-baseline visits (*Incorporation in protocol amendment of Note-to-file from 7 July 2022*)	7.4 (Table 4)
	Minor clarification to definition of trial populations included in the analysis to align with Statistical Analysis Plan	11.2, 11.2.4
	Clarification of contraceptive requirements for male participants to reflect how the protocol was conducted (*Incorporation in protocol amendment of Note-to-file from 7 July 2022*)	16.6
	Inclusion of additional references and correction of error in references	11.1, 11.7, References

TABLE OF CONTENTS

ABBREVIATIONS – GLOSSARY OF TERMS

ADL	Activities of daily living
ADME	Absorption, distribution, metabolism, excretion
AE	Adverse event
ALT	Alanine transaminase
AMR	Antimicrobial resistance
API	Active pharmaceutical ingredient
ART	Anti-retroviral therapy
AUC	Area under the time-concentration curve
BA	Bioavailability
BID	Twice a day
BV	Bacterial vaginosis
CFR	Code of Federal Regulations
CT	*Chlamydia trachomatis*
CI	Confidence interval
CIOMS	Council for International Organizations of Medical Sciences
CKD-EPI	Chronic kidney disease - epidemiology collaboration
CL/F	Apparent total clearance of the drug after oral administration
Cmax	Maximum plasma drug concentration
Cmin	Minimum plasma drug concentration
CRF	Case report form
CTCAE	Common Terminology Criteria for Adverse Events
CV	Coefficient of variation
CYP3A4	Cytochrome P450 family 3 subfamily A member 4
DMID	Division of Microbiology and Infectious Diseases
DNA	Deoxyribonucleic acid
DNDi	Drugs for neglected diseases initiative
DoH	Department of Health
DSMB	Data and safety monitoring board
eCRF	Electronic case report form
EDC	Electronic data capture
eGFR	Estimated glomerular filtration rate
EMA	European Medicines Agency
EOT	End of trial
ESC	Extended spectrum cephalosporines
EU	European Union
FBC	Full blood count
FDA	Food and Drug Administration
FPFV	First participant first visit
fT>MIC	Period over which free drug concentrations exceed MIC
FWA	Federal-wide assurance
g	Gram
GARDP	Global Antibiotics Research & Development Partnership
GCLP	Good clinical laboratorial practice
GCP	Good clinical practice
GI	Gastrointestinal

GM	Geometrical mean
GMP	Good manufacturing practice
GSD	Geometrical standard deviation
H	Hour
HCl	Hydrochloric acid
HFIM	Hollow fibre infection model
HIC	High income country
HIV	Human immunodeficiency virus
HLGT	High level group term
HPLC	High performance liquid chromatography
IB	Investigator brochure
ICF	Informed consent form
ICH	International Conferences on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICMJE	International committee of medical journal editors
IEC	Independent ethics committee
IM	Intramuscular
IMP	Investigational medicinal product
IRB	Independent review board
ITT	Intention to treat
IV	Intravenous
Ke	Elimination rate
Kg	Kilogram
L	Litre
LFT	Liver function tests
LPLV	Last participant last visit
MAR	Missing at random
MDR	Multi drug resistant
MedDRA	Medical Dictionary for Regulatory Activities
mEq	Milli-equivalent
mg	Milligram
MIC	Minimum inhibitory concentration
MIC_{90}	Minimum inhibitory concentration required to inhibit the growth of 90 % of organisms
Micro-ITT	Microbiologic – intention to treat
mL	Millilitre
MNAR	Missing not at random
MoH	Ministry of Health
MOP	Manual of procedures
MoPH	Ministry of Public Health
MS	Mass spectroscopy
µg	Microgram
NAAT	Nucleic acid amplification test
NBTI	Novel bacterial topoisomerase inhibitors
NCE	New chemical entity
ng	Nanogram
NG	*Neisseria gonorrhoeae*

NIH	National Institute of Health
NRA	National regulatory authorities
PCI	Packaging Coordinators Inc.
PD	Pharmacodynamics
PI	Principal investigator (see note Section 13)
PID	Pelvic inflammatory disease
PK	Pharmacokinetics
PO	Per os
PP	Per protocol
PT	Preferred term
PV	Pharmacovigilance
QC	Quality control
QTc	Corrected QT interval
rBA	Relative bioavailability
RDT	Rapid diagnostic test
RNA	Ribonucleic acid
rRNA	Ribosomal ribonucleic acid
S. aureus	Staphylococcus aureus
SAD	Single ascending dose
SAE	Serious adverse event
SAPHRA	South Africa Health Products Regulatory Authority
SD	Standard deviation
SmPC	Summary of product characteristics
SOC	System organ class
SOP	Standard operating procedures
STI	Sexually transmitted infections
SUSAR	Suspected unexpected serious adverse reaction
t1/2	Elimination half life
Thai-FDA	Thailand Food and Drug Administration
t_{last}	Time to last measurable drug concentration following drug administration
t_{max}	Time to reach Cmax following drug administration
TOC	Test of cure
UD	Urethral discharge
ULN	Upper limit of normal
USA	United States of America
US-CDC	United States Centers for Disease Control and Prevention
US-FDA	United States – Food and Drug Administration
VCT	Voluntary counselling and testing
VZ/F	Apparent volume of distribution during terminal phase
USPI	United States Prescribing Information
WBC	White blood cell
WGS	Whole genome sequencing
WHO	World Health Organization
WNL	Within normal limits
wt	weight

PROTOCOL SUMMARY

Trial 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
Protocol number	STI_Zoli001
Phase of development	Phase III
Design	Non-inferiority, parallel, open-label
Primary objective	To assess the efficacy of a single, oral, 3 grams (g) dose of zoliflodacin compared to a combination of a single intramuscular (IM) 500 milligram (mg) dose of ceftriaxone and a single 1 g oral dose of azithromycin for the treatment of uncomplicated urogenital gonorrhoea
Secondary objectives	• 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 gonorrhoea • 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, 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) (day 6 ±2) 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
PK (pharmacokinetics) objective (sub-study only)	To evaluate the plasma PK of a single, oral, 3 g dose of zoliflodacin: • in adult participants (≥ 18 years old) with uncomplicated gonorrhoea who are human immunodeficiency virus (HIV) negative or HIV positive • in HIV negative adolescent participants (≥ 12 and < 18 years old) with uncomplicated gonorrhoea
Exploratory objectives	• To determine the late eradication rate of NG nucleic acids 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) are found positive for NG and identify possible determinants of zoliflodacin resistance
Primary endpoint	Microbiological cure as determined by culture at urethral or cervical sites at TOC (day 6 ±2)
Secondary endpoints	• 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 • 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 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 TOC 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)

Exploratory endpoints	• Out of participants with a positive NG NAAT test and a negative NG culture at TOC, 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 patients 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) of NG isolates from the TOC and end of trial (EOT) visits plus all baseline isolates (urogenital, pharyngeal and rectal) from the

	same patients with possible microbiological failure
Pharmacological endpoints	Plasma PK parameters of zoliflodacin in HIV negative and HIV positive participants (≥ 18 years old) Plasma PK parameters of zoliflodacin in HIV negative adolescents (≥12 and <18 years old)
Inclusion criteria	1. ≥ 12 years old (if enrolment of minors is in agreement with local regulations and ethics guidance) 2. ≥ 35 kilogram (kg) 3. Signs and symptoms consistent with urethral or cervical gonorrhoea OR Urethral or cervical uncomplicated gonorrhoea as determined by either a positive culture or NAAT or Gram stain or methylene blue/gentian violet stain in the past 14 days prior to screening OR Unprotected sexual contact with an individual confirmed to be infected with NG in the past 14 days prior to screening (positive NAAT, Gram stain, methylene blue/gentian violet stain or culture) 4. For females of child-bearing potential, a negative urine pregnancy test at screening 5. For females of child-bearing potential, use of a highly effective method of contraception at the time of IMP administration on Day 1 (see Appendix 1: Contraception methods considered as highly effective and required duration) and until at least 28 days after treatment. Females on oral contraceptives must also use a barrier contraception method during participation in the study. 6. For males with a female partner of child-bearing age, willingness to delay conception during the trial and for 28 days after treatment 7. Willingness to comply with trial protocol 8. For participants in the PK sub-study: Willingness to undergo HIV testing 9. Willingness to abstain from sexual intercourse or use condoms for vaginal, anal and oral sex from enrolment until the end of trial (EOT) visit

10. Willingness and ability to give written informed consent or be consented by a legal representative, or provide assent and parental consent (for minors, as appropriate)

Exclusion criteria

11. Confirmed or suspected complicated or disseminated gonorrhoea
12. Pregnant or breastfeeding females
13. Known concomitant infection which would require immediate additional systemic antibiotics with activity against NG
14. Use of any systemic or intravaginal antibiotics with activity against NG within 30 days prior to screening
15. Use of systemic corticoid drugs or other immunosuppressive therapy within 30 days prior to screening
16. Use of moderate or strong CYP3A4 inducers (e.g. efavirenz, rifampicin, carbamazepine, phenobarbital) within 30 days or five half-lives of the drug, whichever is greater, prior to screening (see Section 6.9)
17. Cytotoxic or radiation therapy within 30 days prior to screening
18. Known chronic renal, hepatic, hematologic impairment or other condition interfering with the absorption, distribution or elimination of the drug, based on medical history and physical examination
19. History of urogenital sex-reassignment surgery
20. Immunosuppression as evidenced by medical history, clinical examination or a recent (≤1 month) CD4 count below 200 cells/microliter (µL)
21. Known clinically relevant cardiac pro-arrhythmic conditions such as cardiac arrhythmia, congenital or documented QT prolongation
22. Known history of severe allergy to cephalosporin, penicillin, monobactams, carbapenems, or macrolide antibiotics
23. Known or suspected allergies or hypersensitivities to lidocaine, methylparaben, lactose or any of the components of the study drugs (refer to the zoliflodacin investigator brochure (IB) and summaries of product characteristics (SmPC) for the comparator treatments)
24. Receipt or planned receipt of an investigational product in a clinical trial within 30 days or five half-lives of the

drug, whichever is greater prior to screening until end of participation to this clinical trial

25. History of alcohol or drug abuse in the 12 months prior to screening which would compromise trial participation in the judgement of the investigator
26. Severe medical or psychiatric condition that, in the opinion of the investigator, may increase the risk associated with trial participation or may interfere with the interpretation of trial results or affect the individual's ability to provide informed consent
27. Individuals whom, in the judgement of the investigator, are unlikely or unable to comply with this trial protocol
28. Previous randomisation in this clinical trial
29. Use of moderate or strong CYP3A4 inhibitors within 30 days or five half-lives of the drug, whichever is greater, prior to screening (see Section 6.9)

Target sample size (estimated)	696 evaluable trial participants (464 in interventional group and 232 in control group) including 20-30 adolescents (≥12 and < 18 years old). The estimated randomised sample size is up to approximately 928 trial participants, and the estimated number of participants enrolled will be approximately 1092 taking into account screen failures.
Intervention	Single oral 3 g dose of zoliflodacin granules as oral suspension
Comparator	Combination of a single intramuscular 500 mg dose of ceftriaxone and a single oral 1 g dose of azithromycin
Statistical analysis	The microbiological intent-to-treat (micro-ITT) population will be used as the primary analysis population for the assessment of microbiological cure. The micro-ITT population will include all randomized participants who receive a dose of drug, who have urogenital NG isolated at baseline and who are not infected with a strain that shows resistance to both ceftriaxone and azithromycin at baseline. The primary efficacy analysis will claim that zoliflodacin is non-inferior to the ceftriaxone-azithromycin combination if the upper bound of the 2-sided 95% confidence interval (CI) for the microbiological cure rate of the combination therapy minus zoliflodacin will be 12% or less (prespecified non-inferiority margin for the primary endpoint).
Complementary activities	Microbiological bacterial isolates collected will be stored for long-term (no longer than 5 years, with the exception of the Netherlands where bacterial isolates will be kept up to 15 years

as per local regulations) after the end of the trial analysis (*e.g*. genotyping) for use in future research in relation to diagnosis and treatment of gonorrhoea.

1. Background and trial rationale

1.1. Background information

Bacterial sexually transmitted infections (STI) are a major public health concern globally, affecting over 357 million people every year (1). *Neisseria gonorrhoeae* (NG), the etiological agent of gonorrhoea, is estimated to have caused 78 million infections in 2012. The Western Pacific and African regions have the highest incidence of gonococcal infections worldwide, with 89 and 50 cases per 100'000 population respectively. Rates of male urethral discharge (UD), which is primarily due to NG or *Chlamydia trachomatis* (CT), are also highest for these two regions, amounting to 567 per 100'000 in Africa and 141 per 100'000 in the Western Pacific. In high income countries (HIC), gonorrhoea is also raising significant concerns. In the United States of America (USA) it is the second most frequently reported notifiable infectious diseases, with over 600'000 infections reported per year (2). NG has been included as one of three organisms presenting an urgent threat due to progressive antimicrobial resistance by the US Centers for Disease Control and Prevention (US CDC) (3).

Gonorrhoea affects both men and women. In men, infections commonly manifest as urethritis. Symptoms develop in 75% of the men within four to eight days of genital infection and in 80 to 90% within two weeks (4). UD is the most frequent presenting symptom and is often undistinguishable from non-gonococcal urethritis (*e.g*. CT infections). Acute unilateral epididymitis can be a complication of gonococcal infection, although it is more common with CT infections (5, 6). In women, gonococcal infections are often (≥50% of the cases) asymptomatic. Genital infections, in particular cervical infections, are the most common infections. When symptomatic, cervical infection typically manifests as mucopurulent discharge. If left untreated, NG infections can ascend to involve the uterus and fallopian tubes. Gonorrhoea is associated with significant morbidity and if not treated appropriately, can have serious consequences on reproductive health. Pelvic inflammatory disease (PID), ectopic pregnancies, abortions, neonatal conjunctivitis and blindness are among the possible complications. Studies have also shown that gonorrhoea enhances the transmission of human HIV by three to five-fold (7-9).

Most individuals with gonorrhoea are managed in the community, and clinical management is often empiric and syndromic: treatment is based on the presence of easily recognized signs (*e.g*. urethral or vaginal discharge), and the provision of antibiotics that deal with the majority of, or the most serious, organisms responsible for the syndrome. Extended spectrum cephalosporins (ESC) constitute the mainstay of gonorrhoea treatment. However, with increasing resistance to ESC emerging globally, and in particular to cefixime, several countries switched around 2013 from ESC monotherapy to a dual therapy that includes an injectable ESC – ceftriaxone- and azithromycin (10, 11), the underlying, unproven, assumption being that a dual therapy would slow down resistance emergence.

Over the past few years, the World Health Organization (WHO) and others have raised concerns over the spread of gonococcal antimicrobial resistance (AMR), warning that infections due to NG may soon become untreatable (12-14). Reports of treatment failures with ESC and azithromycin monotherapy have multiplied over the past few years, and clinical failures to dual therapy have been reported (15-17). Despite this, the drug development pipeline for NG remains insufficient. In 2012, the WHO launched a global action plan to control the spread and impact of AMR in NG. Identifying new treatment options for multi-drug resistant (MDR) gonorrhoea is a key element of the WHO global action plan, and has long been

called for by clinicians and microbiologists (18, 19).

1.2. Zoliflodacin development

Zoliflodacin (ETX0914) belongs to a new class of antibiotics that inhibit bacterial deoxyribonucleic acid (DNA) replication. It shows *in vitro* antibacterial activity against several Gram-positive pathogens such as *Staphylococcus spp*. and *Streptococcus spp*. as well as fastidious Gram-negative pathogens such as *Haemophilus influenzae*, *Moraxella catarrhalis* and NG (20). Minimum inhibitory concentration required to inhibit the growth of 90% of organisms (MIC_{90}) values for NG range from 0.125 to 0.25 µg/mL.

A complete program of both oral and intravenous (IV) toxicity studies was performed in the rat and dog. Data collected included clinical observations, body weight changes, food consumption, clinical pathology and histopathology. Also completed was a battery of genetic toxicology studies and single dose safety pharmacology studies to assess cardiovascular, central nervous system, renal, respiratory and gastrointestinal endpoints.

Studies performed in *the Staphylococcus aureus* (*S. aureus*) neutropenic thigh model, which was the surrogate pathogen model available at the time, indicated that the ratio between the area under the concentration-time curve (AUC) and the MIC was the driver of log kill. Those studies also supported a predicted efficacious mean AUC in humans of 49 µg*hour(h)/mL. Overall, the preclinical data supported progression to phase I trials (20, 21). More recently, dose-ranging studies using a hollow fibre infection model (HFIM) for NG confirmed the exposure-response previously generated with *S. aureus* that, accounting for PK variability in human, zoliflodacin should be administered as a single oral dose of 3g to provide effective killing of NG while suppressing emergence of resistance (22).

Detailed results of these pre-clinical evaluations are summarized in the investigator brochure (IB).

Zoliflodacin is being developed as an oral, single dose treatment. Final data is available from six clinical trials : a phase I single-ascending dose (SAD) trial, a phase I absorption, distribution, metabolism, excretion (ADME) trial (23), a phase II trial involving participants with confirmed urogenital gonococcal infection (24), a phase I relative bioavailability (rBA) trial (clinicaltrials.gov NCT03404167), a phase I food effect trial with healthy volunteers to assess the effect of food on the PK characteristics of a newly developed formulation (clinicaltrials.gov NCT03718806) and a thorough QT/QTc (TQT) trials (25). The SAD and ADME trials showed that zoliflodacin was well tolerated at all doses tested (200 mg- 4’000 mg). The most frequently reported AEs in both healthy volunteers and patients with uncomplicated gonorrhoea were headache, nausea and diarrhea. Dysgeusia, which is the preferred term for ‘unpleasant taste’ in the Medical Dictionary for Regulatory Activities (MedDRA), was reported by healthy volunteers receiving an old zoliflodacin formulation, which is not the formulation used in this trial.

In the phase II, 96% (95%CI: 88-100%) cure rates were observed with both the 2 g and the 3 g doses in the micro-ITT population at the urogenital site (55 out of 57 and 54 out of 56 participants respectively) compared to 100% (95% CI:88-100) in the comparator arm of ceftriaxone 500 mg IM (24). All participants with concomitant rectal infections were cured with either dose of zoliflodacin, while for those with concurrent oropharyngeal infections, cure rates were lower (2 g 50% [95%CI: 16-84]; 3 g 82% [95%CI: 48-98]) compared to 100% (95%CI: 40-100) for ceftriaxone. The small number of participants with concurrent

oropharyngeal infections included in the phase II (8 participants in the 2 g arm, 11 participants in the 3 g arm and 4 in the ceftriaxone arm) however precludes any firm conclusion about efficacy at this site. In the phase II, the safety profile of zoliflodacin was generally similar to ceftriaxone; the most common adverse events (excluding infection-related AEs) were diarrhoea, headache, and nausea. The drug was overall generally well tolerated.

1.3. Overall rationale for the trial

Despite growing concerns over progressive antimicrobial resistance, the development pipeline for uncomplicated gonorrhoea remains relatively empty with only two new chemical entities (NCE) currently in development: zoliflodacin and gepotidacin. Gepotidacin, for which with single dose treatment a few microbiological failures occurred in phase 2, especially in isolates with high baseline MICs (26), is being tested in phase III as a 2-dose treatment for uncomplicated gonorrhoea. As fluoroquinolone resistance is already extremely prevalent globally (27), the use of gepotidacin will have to be restricted to cases where the antimicrobial susceptibility pattern of the infecting strain is known. Zoliflodacin (AZD0914) on the other hand, has shown extremely good activity against a large collection of isolates (standard and clinical), from different geographical origins, including multi-drug resistant (MDR) and extremely drug resistant (XDR) strains (28-31). *In vitro* studies clearly show the absence of cross-resistance between zoliflodacin and pre-existing fluoroquinolone and cephalosporin resistance mechanism (32). The frequency of spontaneous resistance to zoliflodacin *in vitro* was found to be low and comparable to frequencies reported for fluoroquinolones ($1.5x10^{-8}$ to $5.2x10^{-9}$ at 4x MIC) (33-35).

The utility of commercialized readily-available drugs as new treatments for uncomplicated gonorrhoea has been investigated in a few trials. For example, a 4-arm trial (NABOGO) was conducted in the Netherlands to formally evaluate ertapenem, fosfomycin and gentamicin compared to ceftriaxone (36). Preliminary data shows that ertapenem , but not gentamicin nor fosfomycin, was non-inferior to ceftriaxone. Nevertheless, ertapenem must also be administered intramuscularly, like ceftriaxone. In case resistance to the currently recommended therapies reaches the critical threshold of 5% set by WHO and other public health agencies, it could have some utility before a new molecule comes to market. However, it does not provide a long-term solution and have well-known drawbacks as the MIC of ertapenem is affected by the ESC resistance determinants (37). In contrast, zoliflodacin has proven to be an excellent clinical candidate both in terms of efficacy and safety. The drug is well tolerated, can be administered orally and has shown high rates of cure for urethral, cervical and rectal gonococcal infection in phase II (24).

In summary, zoliflodacin is a new promising option to address the risk posed by the threat of drug-resistant gonorrhoea. The purpose of this phase III multi-center, randomized, open-label, non-inferiority trial is to confirm the efficacy and safety outcomes observed in the phase II trial and to generate the necessary evidence to support a marketing authorization application in the USA, the European Union (EU), South Africa and Thailand.

2. Trial objectives and endpoints

Table 1: objectives and endpoints

OBJECTIVES	ENDPOINTS
PRIMARY	
To assess the efficacy 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 for the treatment of uncomplicated urogenital gonorrhoea	Microbiological cure as determined by culture at urethral or cervical sites at TOC (day 6 ±2)
SECONDARY	
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 gonorrhoea	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
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	Proportion of participants with microbiological cure as determined by culture at pharyngeal sites at TOC (day 6 ±2)
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	Proportion of participants with microbiological cure as determined by culture at rectal sites at TOC (day 6 ±2)
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	Proportion of male participants with clinical cure at TOC (day 6 ±2)
To determine the microbiological cure rate of urogenital gonorrhoea among women and men, after administration of a single,	Proportion of female and male participants with microbiological cure as determined by culture at cervical or urethral site at TOC

OBJECTIVES	ENDPOINTS
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	(day 6 ±2)
To determine the microbiological cure rate of 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)	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)
To determine the antimicrobial susceptibility profile of gonococcal strains isolated from participants with uncomplicated gonorrhoea at baseline and the TOC visit	Antimicrobial susceptibility profile of gonococcal strains isolated at baseline and at TOC (day 6 ±2)
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	• Proportion of participants with a negative NG 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)
PK sub-study 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	• 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)

OBJECTIVES	ENDPOINTS
EXPLORATORY	
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	Out of participants with a positive NG NAAT test and a negative NG culture at TOC, 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)
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	Out of participants with symptoms at end of the trial (day 30 +/- 3 days), the following will be determined: • Proportion of patients 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)
To investigate relationships between pre-treatment and post-treatment NG isolates within those participants whose cultures at TOC and EOT are found positive for NG and identify possible determinants of zoliflodacin resistance	WGS of NG isolates from the TOC and EOT visits plus all baseline isolates (urogenital, pharyngeal and rectal) from the same patients with possible microbiological failure

3. Trial design

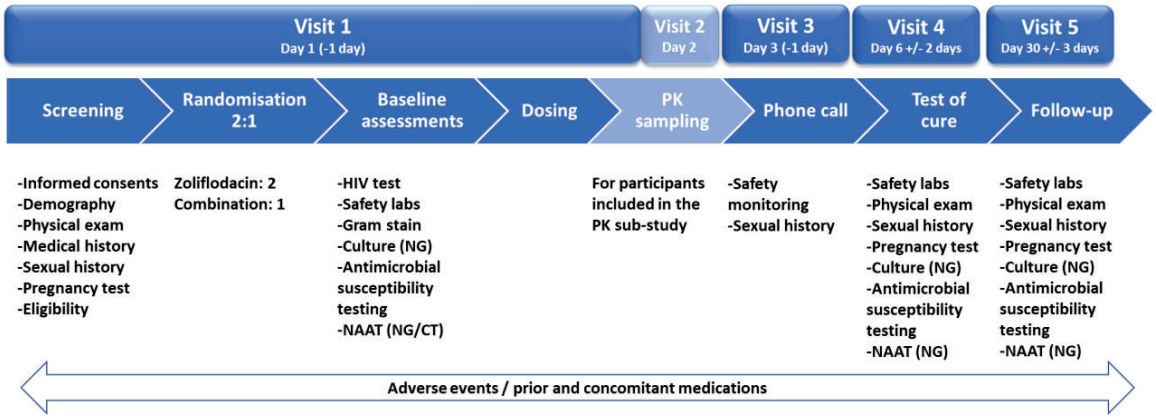
3.1. Design

This trial will be a multi-center, open label, randomized controlled, non-inferiority phase III 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.

A schematic of the trial schedule is presented in Figure 1. Participants presenting to the clinic

will be assessed for eligibility after informed consent is signed on Day 1. 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. Those participants who are assigned to the zoliflodacin group and who will also have consented to be enrolled in the PK sub-study will undergo blood sampling on Day 1 and return to the clinic on Day 2 for further blood sampling. Site staff will call all 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). Those participants that will *a posteriori* have been diagnosed with a CT infection via NAAT at baseline and who were randomised to the zoliflodacin arm will then be treated for the CT infection as per standard of care. All participants will be asked to return to the clinic for the EOT visit on Day 30 for final safety and efficacy assessments. For further details on the schedule of assessments refer to Section 5.

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 (Section 5).

3.2. Rationale

3.2.1. Justification for trial design

The choice of a non-inferiority trial design is based on the fact that an efficacious treatment already exists for uncomplicated gonorrhoea, although not all participants achieve microbiological cure. Given the high cure rate achieved with the ceftriaxone/azithromycin combination (≥95%) it would be unethical to conduct a placebo-controlled trial. It would also be unfeasible to show superiority of zoliflodacin. On the other hand, zoliflodacin offers some advantages over the ceftriaxone/azithromycin combination: it has *in-vitro* activity against azithromycin-, cefixime- and ceftriaxone- resistant strains and it is an oral treatment while ceftriaxone is an injectable treatment. A non-inferiority design is therefore justified and is consistent with the US Food and Drug Administration (FDA) guidance for industry (38). Justification for the non-inferiority margin is available in Section 11.1.

3.2.2. Justification for investigational drug dose selection

Pharmacokinetic/pharmacodynamic (PK/PD) targets have not been developed or established

specifically for NG in animal or human studies. Because of spontaneous eradication of the bacteria, proven adequate animal models of gonococcal infection do not exist and a mouse *S. aureus* neutropenic thigh model was used as a surrogate to determine the human dose for zoliflodacin (20). Based on the *S. aureus* trial, the highest MIC observed to date (0.25 mg/litre [L]) and data from the SAD phase I trial, doses of 2 g and 3 g were investigated in the phase II trial. The key assumption behind dose selection was that the PK/PD index best associated with clinical efficacy was AUC/MIC (20). In the phase II trial, high cure rates were achieved with both a 2 g and a 3 g dose. In the primary analysis of the micro-ITT population, 55 of 57 (96.5%, CI: [87.8%, 99.6%]) infected participants in the zoliflodacin 2 g arm achieved microbiological cure at the urethral/cervical site. Of the 56 infected participants in the zoliflodacin 3 g arm, 54 (96.4%, CI: [87.7%, 99.6%]) achieved microbiological cure and of the 28 infected participants in the ceftriaxone arm, all 28 (100%, CI: [87.7%, 100%]) achieved microbiological cure. In the secondary analysis of the per protocol (PP) population, 48 of 49 (98.0%, CI: [89.2%, 99.9%]) infected participants in the zoliflodacin 2 g arm achieved microbiological cure at the urethral/cervical site. Of the 47 infected participants in the zoliflodacin 3 g arm, all 47 (100%, CI: [92.5%, 100%]) achieved microbiological cure. Of the 21 infected participants in the ceftriaxone arm, all 21 (100%, CI: [83.9%, 100%]) achieved microbiological cure. Microbiological cures at the rectal and pharyngeal sites were also investigated in the Phase II trial. Further details are available in the IB. The phase II trial used a compounded suspension of active pharmaceutical ingredient (API) in an aqueous vehicle containing standard excipients. This formulation was not commercially viable, hence a granule for oral suspension formulation has been developed for further testing in clinical trials and commercialization. The rBA trial (clinicaltrials.gov NCT03404167) and the final data from the food effect trial (Clinicaltrials.gov NCT 03718806) showed that the exposure achieved with granule formulation is comparable to that achieved with the aqueous suspension formulation. The PK characteristics measured during these trials may be found in the IB.

PK/PD modelling suggests that a 3 g dose of the granule formulation will result in an AUC of 30500 ng•h/mL (5%-95% quantile bounds: 9960 - 78400 ng•h/mL) and 53600 ng•h/mL (5%-95% quantile bounds: 22400 - 107000 ng•h/mL) in fasted and fed subjects respectively. These values are comparable to the AUC achieved with a 3 g dose of the previous aqueous suspension formulation. Additional information can be found in the IB.

Based on those results, to maximize cure rates both at urogenital and extra-genital sites and to cover isolates with higher MICs, a 3 g dose was selected as the final dose for the phase III trial. This dose was well tolerated in both phase I and II studies. To further support a 3 g dose, results of dose-ranging HFIM experiments with NG recently confirmed that, accounting for PK variability in human, a 3 g dose provides effective killing of NG while suppressing emergence of resistance (22). It thus appears that the 3 g dose provides the best benefit/risk ratio.

3.2.3. Justification for comparator selection

The present trial will be the first global trial investigating a new antimicrobial that aims at addressing a global public health need. The Global Antibiotic Research and Development Partnership (GARDP) overarching goal is to conduct a single phase III trial that meets the regulatory requirements of the US FDA, the South African Health Products Regulatory Authority (SAHPRA), the Thai Food and Drug Administration (Thai FDA) and the European Medicines Agency (EMA), and that enables registration and roll out of zoliflodacin in those countries. However, a major challenge is to choose a unique comparator against which zoliflodacin can be evaluated in a scientifically robust way.

The comparator for this trial is a single combination of an IM dose of 500 mg ceftriaxone and a 1 g oral dose of azithromycin. The rationale for use of this combination is to compare zoliflodacin to the first line treatment recommended by most current national and international guidelines (see Table 2). With regards to the dose of ceftriaxone, a 500 mg dose was chosen for the following reasons:

1) Dosages of ceftriaxone up to 1 g have proven safe and are approved for use in various indications by all regulatory agencies cited above. A dose of 500 mg ceftriaxone is recommended for first line treatment of uncomplicated gonorrhoea in the US and the EU and for re-treatment/treatment failure by WHO guidelines for the treatment of NG (39-41).
2) There is currently no firm clinical evidence to support (or to oppose) a 500 mg versus a 250 mg dose of ceftriaxone for treatment of gonorrhoea. However, modelling data suggests a higher dose may achieve higher log kill, in particular in situation where MICs are in the upper range (40, 42). It is generally assumed that the clinical efficacy of β-lactam antibiotics relates to *f*T > MIC and experiments conducted by Jaffe and colleagues found that penicillin efficacy in male urethritis was predicted by total (*i.e*. serum-bound plus free) penicillin levels at least four times above the MIC for at least 10 h, roughly corresponding to an fT > MIC of 7–10 h (43). For cephalosporins, Chisholm et al. postulated that an fT > MIC of 20–24 h is needed for efficacy with cephalosporins (42). This is unlikely to be achieved by a 250 mg dose while it is very likely by a 500 mg dose.
3) At the moment the ceftriaxone MICs are still largely within the susceptible range (≤0.125 µg/ml) and 250 mg dose is likely to achieve sufficient exposure to cover most isolates. However, MIC "creep" is being observed in several countries, including in South Africa and Thailand, and reports of treatment failures have been published from several countries internationally (28, 44, 45). In the WHO Southeast Asian Region, decreased susceptibility or resistance to ceftriaxone was noted by 50% (3/6) of the countries (Bhutan, India, and Indonesia) reporting ceftriaxone susceptibility data in 2014; 33% (2/6; India and Indonesia) reported ≥ 5% decreased susceptibility or resistance (27). From 2009 to 2014, 83% (5/6) of countries reported isolates with decreased susceptibility or resistance to ceftriaxone, and 50% (3/6) reported ≥ 5% decreased susceptibility or resistance (27). The latest estimates from China also show that between 9.7% and 12.2% of the isolates have decreased susceptibility to ESC (46). In essence, this means that by the time zoliflodacin is licensed it is likely that the WHO-recommended dosage of ceftriaxone will have also increased. Several countries already use 500 mg to 1 g of ceftriaxone (*e.g*. China, Germany, The Netherlands, Japan and the US). Comparing zoliflodacin to 500 mg dose of ceftriaxone during the phase III will therefore bring more meaningful data to support registration.
4) Based on the assumption above, showing non-inferiority of zoliflodacin to 500 mg of ceftriaxone + 1 g azithromycin will be more conservative, as well as more widely applicable than if a 250 mg ceftriaxone dose + 1 g azithromycin was used as a comparator.
5) This dosage was used in other gonorrhoea trials, including the zoliflodacin phase II trial, and achieved high cure rates and excellent safety profile.
6) The combination of ceftriaxone with azithromycin is the standard of care in many countries (See Table 2).

Table 2: Summary of currently recommended regimens for the treatment of uncomplicated gonorrhoea

	Uncomplicated urogenital infections due to NG	Uncomplicated urogenital infections due to CT	Reference
WHO	250 mg ceftriaxone, IM, single dose + 1 g azithromycin, oral, single dose Or 400 mg cefixime[1] oral single dose +1 g azithromycin, oral, single dose	1 g azithromycin, oral, single dose Or 100 mg doxycycline, oral, twice a day (BID) for 7 days	(39, 47)
US CDC	From December 2020: 500 mg ceftriaxone, IM, single dose	From December 2020: 100 mg doxycycline, oral, BID for 7 days	(40)
	Before December 2020: 250 mg ceftriaxone, IM, single dose + 1 g azithromycin, oral, single dose	Before December 2020: 1 g azithromycin oral, single dose or 100 mg doxycycline, oral, BID for 7 days	(48) (49)
South Africa national guidelines	No differentiation between NG and CT infections 250 mg ceftriaxone + 1 g azithromycin		
Thailand national guidelines	500 mg ceftriaxone, IM, single dose Or 400 mg cefixime[1,2] oral, single dose	1 g azithromycin, oral, single dose Or 100 mg doxycycline, oral, BID for 14 days	(50)
Europe guidelines	500 mg ceftriaxone, IM, single dose + 2 g azithromycin oral, single dose	1 g azithromycin oral, single dose Or 100 mg doxycycline, oral, BID for 7 days	(41)
Netherlands guidelines	500 mg ceftriaxone, IM, single dose	1 g azithromycin, oral, single dose Or 100 mg doxycycline, oral, BID for 7 days	(51)

CT: *Chlamydia trachomatis*; IM: intramuscular; NG: *Neisseria gonorrhoeae*; US-CDC: United States Centers for Disease Control and Prevention; WHO: World health Organisation.

1. Provided recent local resistance data shows susceptibility to the antibiotic
2. Azithromycin is not a requirement for treatment of uncomplicated gonorrhoea but recommended for the empiric treatment of symptomatic individuals

3.2.4. Justification for exclusion of CYP3A4 inhibitors

In the initial development of zoliflodacin a physiologically-based PK (PBPK) model incorporating physicochemical properties, absorption, distribution, metabolism, and excretion of zoliflodacin was developed to investigate the systemic exposure of the drug after oral administration to healthy adults and determine the potential for drug-drug interactions (DDIs) (see IB for details). Based on the output of these simulations using data derived from the original amorphous formulation, the totality of the data indicated that zoliflodacin has low potential for significant DDIs across a broad range of CYP P450s and transporters except CYP3A4. Strong CYP3A4 inhibitors were predicted to have a modest impact on zoliflodacin (~1.3 fold increase on Cmax and a 2.4 - 2.6 fold increase in AUC) while moderate to strong CYP3A4 inducers have the potential to reduce zoliflodacin exposure below what is required for efficacy.

The increase in AUC predicted by physiologically-based PK model in zoliflodacin in the presence of strong inhibitors (ketoconazole and ritonavir) was used directly to adjust the typical value estimate of clearance (tvCL) in the population PK model. The population PK model was then used to conduct Monte Carlo simulations of a virtual patient population. In this manner the potential effect of a strong CYP3A4 inhibitor on the simulated PK could be considered across fed/fasted status and body weight range.

In light of the substantive food effect observed in the recent Phase I Food Effect study (DNDi/GARDP STI_Zoli002) of the new immediate release formulation ("Phase III formulation"), the Sponsor has undertaken further in silico evaluation of the potential impact of CYP3A4 inhibition on zoliflodacin exposure using the refined population PK model used to extrapolate exposure in lower body weight ranges of 35 to 50 kg; for these analyses, the potential effect of a strong CYP3A4 inhibitor on zoliflodacin concentrations was considered by reducing the tvCL value in the model by 2.37 and 2.61 fold based upon mean predicted fold changes in AUC (derived from SimCYP simulations) with ketoconazole and ritonavir as inhibitors.

Output of Monte Carlo simulations for a 3000 mg dose utilizing these predicted fold changes in AUC of zoliflodacin in the presence of each strong CYP3A4 inhibitor suggested the highest mean Cmax achieved in the lowest bodyweight of 35 kg (weight cut-off limit in the ongoing Phase III study GARDP STI_Zoli001), would be nearly equivalent to the maximum concentration that has been observed clinically; i.e. mean geometric mean Cmax of 37.5 µg/mL in subjects administered a 4000 mg (“Phase III formulation”) with a high fat meal.

Based on this analysis, the Sponsor concluded that it was prudent to prohibit concomitant dosing of zoliflodacin with moderate/strong CYP3A4 inhibitors until further clinical and PK data are available from PK subset groups in the present Phase III study and from a planned study that is investigating drug-drug interactions and food effect (STIZoli_003, EudraCT number 2020-000379-19).

3.2.5. Impact of COVID-19 pandemic on trial conduct and contingency measures

The COVID-19 pandemic is providing unprecedented challenges to all healthcare workers and patients in general and for recruitment and running of clinical trials.

GARDP therefore took, and continues to take, contingency measures to mitigate the impact of the pandemic on the trial, to support site staff and patients, and to minimise risks to

them, while being able to continue recruitment to the trial and to guarantee data quality and integrity.

Contingency measures are described in a separate COVID-19 mitigation plan.

3.3. Randomization

The randomization sequence will be obtained by computer-generated random numbers and provided to each trial site through a web-based randomization system. 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. Randomisation will be performed using random permutated blocks of 3, 6, and 9 with stratification by sex at birth and with each block maintaining the 2:1 randomisation. 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.

3.4. Blinding

This will be an open-label trial. 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.

Nevertheless, 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 electronic case report form (eCRF) by site staff, or reported directly to data management as per pre-determined data specifications. For sites participating in the PK sub-study, 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).

3.5. Trial duration

The planned total duration for individual participants will be 30 days (± 3 days). 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 the trial is defined as the last participant's last visit. Overall and taking into consideration an evaluation of the impact of the COVID-19 pandemic on recruitment, recruitment into the trial is expected to last for approximately 3.5 years.

4. Selection of participants

The target sample size is 696 evaluable participants: 464 in the zoliflodacin group and 232 in the comparator group, with an estimated randomisation sample size of up to approximately 928. Details of the sample size calculation can be found in Section 11.1. Accounting for anticipated screening failures, the enrolment sample size is estimated to be around 1092.

Only participants who meet **ALL** inclusion criteria and **NONE** of the exclusion criteria will be eligible for enrolment into the trial. All relevant medical and non-medical conditions should be taken into consideration when deciding whether this protocol is suitable for a particular participant. Eligibility criteria must not be waived by the investigator nor by the sponsor.

4.1. Inclusion criteria

Participants must meet **ALL** the following inclusion criteria to be eligible for enrollment into the trial:

1. Age ≥ 12 years old (if enrolment of minors is in agreement with local regulations and ethics guidance)
2. Weight ≥ 35 kg
3. Signs and symptoms consistent with urethral or cervical gonorrhoea

   OR

   Urethral or cervical uncomplicated gonorrhoea as determined by either a positive culture or NAAT or Gram stain or methylene blue/gentian violet stain in the past 14 days prior to screening

   OR

   Unprotected sexual contact with an individual reported to be infected with NG in the past 14 days prior to screening (confirmation by a positive NAAT, Gram stain, methylene blue/gentian violet stain or culture)
4. For females of child-bearing potential, a negative urine pregnancy test at screening
5. For females of child-bearing potential, use of a highly effective method of contraception at the time of IMP administration on Day 1 (see Appendix 1: Contraception methods considered as highly effective and required duration) and during at least 28 days after treatment. Females on oral contraceptives must also use a barrier contraception method during participation in the trial
6. For males with a female partner of child-bearing age, willingness to delay conception for 28 days after treatment
7. Willingness to comply with trial protocol
8. For participants in PK sub-study: Willingness to undergo HIV testing
9. Willingness to abstain from sexual intercourse or use condoms for vaginal, anal and oral sex until the EOT visit
10. Willingness and ability to give written informed consent or be consented by a legal representative or provide assent and parental consent (for minors, as appropriate).

4.2. Exclusion criteria

The presence of any of the following will exclude a potential participant from trial enrolment:

11. Confirmed or suspected complicated or disseminated gonorrhoea
12. Pregnant or breastfeeding women
13. Known concomitant infection which would require immediate additional systemic antibiotics with activity against NG
14. Use of any systemic or intravaginal antibiotics with activity against NG within 30 days prior to screening
15. Use of systemic corticoid drugs or other immunosuppressive therapy within 30 days prior to screening
16. Use of moderate or strong CYP3A4 inducers (e.g. efavirenz, rifampicin, carbamazepine, phenobarbital) within 30 days or five half-lives of the drug, whichever is greater, prior to screening (see Section 6.9)
17. Cytotoxic or radiation therapy within 30 days prior to screening
18. Known chronic renal, hepatic, hematologic impairment or other condition interfering with the absorption, distribution or elimination of the drug based on medical history and physical examination
19. History of urogenital sex-reassignment surgery
20. Immunosuppression as evidenced by medical history, clinical examination or a recent (≤ 1 month) CD4 count <200 cells/µL
21. Know clinically relevant cardiac pro-arrhythmic conditions such as cardiac arrhythmia, congenital or documented QT prolongation
22. Known history of severe allergy to cephalosporin, penicillin, monobactams, carbapenems or macrolide antibiotics
23. Known or suspected allergies or hypersensitivities to lidocaine, methylparaben, lactose or any of the components of the study drugs (refer to the zoliflodacin IB and SmPC for the comparators treatments)
24. Receipt or planned receipt of an investigational product in a clinical trial within 30 days or five half-lives of the drug, whichever is greater, prior to screening until end of participation to this clinical trial
25. History of alcohol or drug abuse within 12 months prior to screening which would compromise trial participation in the judgment of the investigator
26. Severe medical or psychiatric condition which, in the opinion of the investigator, may increase the risk associated with trial participation or may interfere with the interpretation of trial results or affect the individual's ability to provide informed consent
27. Individuals whom, in the judgement of the investigator, are unlikely or unable to comply with this trial protocol
28. Previous randomisation in this clinical trial
29. Use of moderate or strong CYP3A4 inhibitors (see manual of operating procedures [MOP]) within 30 days or five half-lives of the drug, whichever is greater, prior to screening (see Section 6.9)

5. Schedule of events

5.1. Table of assessments

The trial investigational schedule is shown in Table 3.

Table 3: Trial investigational schedule

Visit number	**1**[1]			**2**	**3**	**4**	**5**	
Timing (window)	**Day 1 (- 1 day)**	**Day 1**		**Day 2**	**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 (EOT)**	**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]	
Antimicrobial susceptibility testing[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	←----------							----------🕓

CT: *Chlamydia trachomatis;* HIV: human immunodeficiency virus; 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 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. 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 Section 5.3 for details.
4. Including counselling on sexual risk behaviour, and partner(s) notification and treatment
5. Including date of birth, gender, sex at birth, ethnicity
6. Using sexual history questionnaires provided
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 NG
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 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.

5.2. Schedule of assessments

Changes in health and concomitant medications will be recorded from signature of the informed consent until the EOT visit. See Section 8 for further details on adverse events.

5.2.1. Visit 1: Day 1 (-1 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.

Screening Day 1 (-1 day)

After signing the informed consent, participants will be asked demographic information, including date of birth (only year of birth and age will be reported in the eCRF), gender, sex at birth and ethnicity. Eligibility criteria will be reviewed, and a complete medical history, and sexual history over the past 3 months will be obtained as part of this process. A targeted physical examination will be performed (the physical examination performed at screening will be regarded as the baseline), including a clinical assessment of gonorrhoea, as well as a urine pregnancy test for females of child-bearing potential.

For participants taking part in the PK sub-study, randomization can occur on Day -1 (Screening) once eligibility has been confirmed, and participants can return to complete Day 1 baseline assessments and receive study treatment the following calendar day, to assist with scheduling of the visit.

Baseline (Day 1)

Eligible participants will be randomised to either the zoliflodacin or the comparator group. They will then undergo baseline assessments, including safety laboratory tests and HIV rapid testing if applicable. 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 do not require repeat testing. Swabs will also be taken for microbiology outcomes (1 swab for NG culture and 1 swab for NG/CT NAAT evaluations) from the urethra or cervix, rectum and pharynx.

Absorption of zoliflodacin and tolerability of azithromycin tablets are improved when patients are fed, and not fasted. Therefore, 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. The date and time of last food intake prior to trial treatment administration will be captured.

Once ALL baseline assessments are completed, participants will receive trial treatment.

Blood samples for PK analysis will be taken as per schedule described in Section 7.3 from those participants who will have signed the PK sub-study informed consent.

5.2.2. Visit 2: Day 2 - PK sub-study

Visit 2 is for participants enrolled in the PK sub-study only. See Section 7.3 for further details.

5.2.3. Visit 3: Day 3 (-1 day)

On day 3, the medical staff will call participants on the phone to enquire about their health condition and sexual history since the last visit. This day is not a clinic visit.

5.2.4. Visit 4: Day 6 (+/-2 days)

At the TOC visit, participants will undergo safety lab assessments, a physical examination including a clinical assessment of gonorrhoea, and females of child-bearing potential will perform a urine pregnancy test. Males at birth will be assessed for clinical cure as per guidance in the MOP. Sexual history since the previous visit will also be assessed. The medical staff will enquire with the participant whether they experienced any potential AE and took any medication since the last visit.

Swabs will be collected for microbiology outcomes (1 swab for NG culture and 1 swab for NG NAAT evaluations) from each anatomical site (urogenital, rectal and pharyngeal), unless both NG culture and NG NAAT results at that specific anatomical site were negative at baseline.

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.

Once all specimens are collected, participants randomised to the zoliflodacin arm with a positive CT NAAT at baseline will be treated for CT infection as per local standard of care.

If a participant returns for a TOC visit outside the allowed time window, the primary TOC assessments will be considered missed and the participant will therefore be regarded as a treatment failure for primary endpoint analysis. It is therefore of crucial importance to ensure all participants have a TOC visit within the allowed time window (Day 6 +/- 2 days).

5.2.5. Visit 5: Day 30 (+/-3 days)

At the EOT visit, participants will undergo safety lab assessments, a physical examination including a clinical assessment of gonorrhoea, and females of child-bearing potential will perform a urine pregnancy test. Sexual history since the previous visit will also be assessed. The medical staff will enquire with the participant whether they experienced any potential AE and took any medication since the last visit.

Swabs will be collected for microbiology outcomes as per Table 4 in Section 7.4.

5.2.6. Early withdrawal

In case a participant is withdrawn or withdraws consent prior to Visit 5 (EOT), they will be asked to return to the clinic to undergo safety lab assessments, a physical examination and a urine pregnancy test if they are a female of child-bearing potential, as well as the sexual history questionnaire, should they agree. The medical staff will enquire with the participant whether they experienced any potential AE and took any medication since the last visit.

If a participant withdraws during the timeframe for Visit 4 or Visit 5, trial staff should attempt to collect samples as per the schedule of assessment at that visit, should the participant agree.

5.3. Unscheduled and out-of-window visits

5.3.1. Out-of-window visits

Protocol-specified visits that occur outside of the protocol-specified time windows will be considered out-of-window visits. Results of assessments and procedures for these should be documented in the eCRF pages for the intended visit and a protocol deviation must be documented. In case of doubt or question on which visit should be performed, the Investigator should contact the medical monitor.

5.3.2. Unscheduled visits

Unscheduled visits can occur at any time during study participation. Assessments are not mandated per protocol but 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. No assessments are mandated as per protocol, but can be performed and recorded for safety reasons as relevant to the study (e.g. follow-up safety laboratory tests). Site staff will take the opportunity to question the participant about any potential adverse event or medication taken. In case of identification of either, this must be recorded in source notes and transcribed into the eCRF into the adverse event and concomitant medication forms respectively.

6. Investigational products

6.1. Zoliflodacin treatment

6.1.1. Product description and dosage

Zoliflodacin is a first-in-class drug (spiropyrimidinetrione) with a specific mode of action against bacterial topoisomerase II distinct from the fluoroquinolone class of antibiotics. The chemical name for zoliflodacin is (2R,4S,4aS)-11-fluoro-2,4-dimethyl-8-[(4S)-4-methyl-2-oxo-1,3-oxazolidin-3-yl]-1,2,4,4a-tetrahydro-2'H,6H-spiro[1,4-oxazino[4,3-a][1,2]oxazolo[4,5-g] quinoline-5,5'-pyrimidine]-2',4',6'(1'H,3'H)-trione. Zoliflodacin is presented as white granules for oral suspension, packaged in a single sachet. Each sachet contains 3 g amorphous zoliflodacin, co-formulated with mannitol, microcrystalline cellulose, croscarmellose sodium, colloidal silicon dioxide, xanthan gum, talc, and magnesium stearate. Zoliflodacin is active against several STI pathogens, including NG, CT and *Mycoplasma genitalium* (33, 52). It shows excellent *in vitro* activity against all clinical NG isolates tested so far, including isolates that were resistant to fluoroquinolones, azithromycin, and ESC, and several reference strains such as the XDR H401 strain (28, 32, 53).

6.1.2. Summary of non-clinical and clinical experience

Please refer to the zoliflodacin IB for full descriptions of the zoliflodacin properties, non-clinical and clinical data available to date and identified and potential risks associated with zoliflodacin.

6.2. Comparator trial treatment

6.2.1. Product description and dosage

Ceftriaxone is a sterile, semi-synthetic, broad-spectrum cephalosporin antibiotic. The chemical name of ceftriaxone sodium is (6R, 7R)-7-[2-(2-Amino-4-thiazolyl) glyoxylamido]-8-oxo-3-[[(1,2,5,6-tetrahydro-2-methyl-5,6-dioxo-as-triazin-3-yl)thio]methyl]-5-thia-1 azabicyclo [4.2.0]oct-2-ene-2-carboxylic acid, 72-(Z)-(O-methyloxime), disodium salt, sesquaterhydrate. The bactericidal activity of ceftriaxone results from the inhibition of cell wall synthesis and is mediated through binding to penicillin binding proteins. Ceftriaxone is a white to yellowish sterile powder in a clear glass vial. For the purpose of this trial, a single dose of ceftriaxone 500 mg, dissolved in lidocaine hydrochloride (HCl) 1% will be used for IM injection. Lidocaine is a local anaesthetic that will be used to numb the area of injection and therefore limit injection pain.

Azithromycin is a macrolide antibacterial, for oral administration. The chemical name of azithromycin monohydrate is (2R,3S,4R,5R,8R,10R,11R,12S,13S,14R)-13-[(2,6-dideoxy-3-C-methyl-3-O-methyl-α-L-ribohexopyranosyl) oxy]-2-ethyl- 3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6- trideoxy-3-(dimethylamino)-β-D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one monohydrate. Azithromycin acts by binding to the 23S ribosomal ribonucleic acid (rRNA) of the 50S ribosomal subunit of susceptible microorganisms inhibiting bacterial protein synthesis and impeding the assembly of the 50S ribosomal subunit. Azithromycin will be supplied as oral tablets containing 250 mg azithromycin.

6.2.1. Summary of non-clinical and clinical experience

Please refer to the ceftriaxone and azithromycin summaries of product characteristics (SmPC) or United States Prescribing Information (USPI), as applicable, for full descriptions of the identified and potential risks associated with ceftriaxone and azithromycin.

6.3. Labelling and packaging

The labelling and secondary packaging of zoliflodacin sachets and comparators treatments will be performed by Packaging Coordinators Inc. (PCI) Pharma Services on behalf of the sponsor. All trial treatments, including those used in the comparator group, will be labelled in compliance with applicable regulatory requirements.

6.4. Shipment

All necessary documentation, including favourable opinion from independent review boards (IRB) /independent ethics committees (IEC) and national regulatory authorities (NRAs), and import authorizations, will be obtained prior to shipment of trial treatments to trial sites.

Upon receipt, trial treatments and their packaging shall be inspected by the pharmacist or designee in charge at the trial site. All suspected defects or deterioration are to be reported to the sponsor as per the procedure outlined in the MOP. Suspected defective products are to be placed in a secure quarantine area pending investigation and further advice from the sponsor.

In the event of a batch recall, the sponsor will be responsible for informing all principal investigators and, in collaboration with trial site pharmacists, of facilitating collection of recalled batches and delivery of alternative batches to trial sites. All movements will be documented in the drug accountability forms (see Section 6.7).

6.5. Storage

All trial treatments will be stored in the trial site pharmacy, separated and secured, accessible only to the trial site staff. All drugs should be stored in accordance with the pharmacy manual.

The trial site pharmacist or designee will be responsible for monitoring and recording the storage temperature and ensuring appropriate measures are in place to protect the trial treatments from damage and/or destruction.

6.6. Preparation and administration of trial treatments

Trial treatments will only be dispensed and administered to individuals enrolled in the trial and who have been randomised. Participants randomized to the zoliflodacin group will receive a single 3 g per os (PO) dose (one sachet) of zoliflodacin reconstituted with water. Detailed information on the preparation and administration of zoliflodacin can be found in the pharmacy manual. Participants randomized to the comparator group will receive ceftriaxone as a 500 mg IM injection and azithromycin as four 250 mg oral tablets. The 500 mg ceftriaxone vial will be reconstituted with lidocaine HCl 1%. Detailed information on the preparation and administration of ceftriaxone can be found in the pharmacy manual.

6.7. Accountability

The principal investigator at each site is responsible for the distribution and disposition of the trial treatments and has ultimate responsibility for accountability of trial treatments. He/she may delegate this responsibility to a trial site pharmacist or nurse. Adequate records on receipt, use, return, loss, or other disposition of trial treatments must be maintained. Administration of trial treatments will be directly observed and documented in appropriate forms by the responsible trial staff. The investigator or site staff are not authorised to supply trial treatment to other investigators or sites, or allow trial treatments to be used other than as instructed by this protocol without prior written authorisation of GARDP. At the end of the trial, unused drugs will be either be destroyed or returned as per sponsor instruction.

6.8. Blinding and procedures for un-blinding

As this is an open-label trial, procedures for un-blinding by the investigator in case of medical emergency are not applicable.

6.9. Concomitant treatments

Medications taken before or after dosing through EOT visit or early termination whichever occurs first will be reported as prior medications or concomitant medications, respectively. Prior medication taken over the 30 days prior to screening will be recorded as part of the screening process (Visit 1). This will also include over-the-counter drugs as well as vitamins, herbals and supplements.

Zoliflodacin should not be co-administered with strong (such as carbamazepine, enzalutamine, mitotane, phenytoin, rifampin, or St. John's wort) or moderate (such as efavirenz, bosentan, etravirine, modafinil, or nafcillin) inducers of CYP3A4. These drugs should not be taken for 30 days before dosing and during the whole trial period.

Zoliflodacin should not be co-administered with strong or moderate inhibitors of CYP3A4. See MOP for examples of such inhibitors. These drugs should not be taken for 30 days before dosing and during the whole trial period.

If a participant is newly diagnosed as HIV positive in the trial and needs to start ART as per

local standard of care, or if a participant needs post exposure prophylaxis with a prohibited ART, the medical monitor should be contacted immediately to discuss each instance on a case-by-case basis. Depending on the timing of the required administration of the ART, its administration may either be approved, or the participant may be withdrawn from the trial.

In addition, medications that might interfere with the evaluation of the investigational product (e.g. antibiotics with activity against NG) should not be used until the TOC visit. Any circumstances that require urgent use of an antibiotic with activity against NG should be discussed with the medical monitor.

Following dosing, any drug (*e.g*., non-prescription medications, herbal supplements, vitamins, or prescription medications) or vaccine or blood/blood products used by the participant during the trial will be recorded in the participant's source documents and on the appropriate eCRF.

Any antacids if taken must be administered more than 1 hour before administration of azithromycin.

Participants who receive trial treatment and are subsequently diagnosed with a concomitant infection requiring systemic antibiotics (*e.g*. bacterial vaginosis [BV]) or infections due to CT, *Mycoplasma genitalium* or *Trichomonas vaginalis*) will receive treatment according to the local clinic's standard protocols. Whenever possible, additional treatment with antibiotics with activity against NG should be deferred up until the TOC visit.

6.10. Escape treatments

Participants with persisting signs and symptoms or with a positive NG culture at the TOC visit will be treated in accordance with national or local guidelines for treatment failures. Treatment received will be recorded in the eCRF.

7. Trial procedures/evaluations

Detailed instructions on procedures as well as collection, processing and shipment of samples may be found in the laboratory manual and the MOP.

7.1. Clinical evaluations

- Complete medical history will be obtained by interview of the participants at Visit 1 and will be updated at each clinic visit. Participants will be queried regarding a history of significant medical disorders of the head, eyes, ears, nose, throat, mouth, cardiovascular system, lungs, gastrointestinal tract, liver, pancreas, kidney, urologic system, nervous system, blood, lymph nodes, endocrine system, musculoskeletal system, skin, and genital/reproductive tract. A history of any allergies, cancer, immunodeficiency, psychiatric illness, substance abuse, and autoimmune disease will be solicited. At follow-up visits, an interim medical history will be obtained by interview of the participants noting any changes since the previous visit.
- Sexual history over the past 3 months including sexual orientation will be obtained by interview of the participants at Visit 1. Participants will be queried at Visit 3, Visit 4 and Visit 5 regarding sexual activity and condom use since the previous clinic visit. Questionnaires to assess sexual behaviour will be provided.
- A targeted physical examination including height and weight and examination of the pharynx and genitals (including pelvic exam for women) will be performed on Visit 1, Visit

4 and Visit 5. In particular, presence of signs and symptoms of gonorrhoea (*e.g*. UD, dysuria, dyspareunia, visible discharge from urethra and/or anus) will be assessed as per the MOP. All physical examinations will be performed by a trial clinician licensed to make medical diagnoses. The physical examination performed at screening will act as a baseline for subsequent visits .

- A phone interview will be conducted at Visit 3 to check for any changes in health and any new medication taken. Participants will also be asked about their sexual activity since the previous visit and be reminded about the mandatory sexual behaviour recommendations during their involvement in the trial.
- Any changes in health or concomitant medications taken during the trial will be enquired and recorded.

7.2. Laboratory evaluations

7.2.1. Clinical laboratory evaluations

- A urine pregnancy test will be performed at Visit 1, Visit 4, Visit 5 and early withdrawal on all female participants of child-bearing potential.
- HIV testing will be performed at Visit 1 according to standard local practise. HIV testing is mandatory only for participants to the PK sub-study. For all other participants, HIV testing is proposed, but not mandatory. Participants with a documented positive HIV diagnosis or documented prescription for ARTs are considered to be HIV positive and therefore do not need to undergo a test. A maximum of 5 ml of venous blood might be collected for this test depending on local practice. Any discordant results should be followed up as per standard local practice. Further details may be found in the MOP.
- Venous blood samples (a maximum of 15 mL) for haematology and chemistry will be collected at Visit 1, Visit 4 and Visit 5. FBC, ALT, direct and total bilirubin (direct bilirubin only required if total bilirubin is elevated), and creatinine will be measured. eGFR will be calculated using the chronic kidney disease - epidemiology collaboration (CKD-EPI) formula. A total volume of a maximum of 50 ml will be taken from each participant during the course of the trial (with the exclusion of the PK sub-study).

7.2.2. Microbiological evaluations

- Microbiological evaluations (Gram stain, culture and NAAT) will be based on the collection of appropriate clinical specimen (*i.e*. a urethral swab for male participants or an endocervical swab for female participants for urogenital swabs, a pharyngeal swab or a rectal swab) and in accordance with the laboratory manual and MOP. Specimens are collected by trained study staff, except pharyngeal specimens which can be self-collected, where permitted and as detailed in the MOP. A specific swab for NG culture will be taken followed by bedside inoculation of NG selective agar plates.
- Assessment of efficacy in the present trial follows recommendations from the US FDA (38). All cultures will be performed in certified national or state/provincial or local reference laboratories (see the laboratory manual for a complete list). Local laboratories will perform presumptive NG identification, while confirmatory NG identification will be carried out by the central laboratory also assessing antimicrobial susceptibility testing.
- Detection of nucleic acid will be based on collection of swabs as above and use of NAAT.

A single swab using the applicable manufacturer's recommended collection kits will be taken to assess the presence of NG and/or CT from the same clinical specimen to minimise patient discomfort. Acceptable NAAT platforms/systems and assays are listed in the laboratory manual. Detailed methods for NAAT performance can be found in the trial laboratory manual.

- Presumed positive cultures will be processed and frozen bacterial isolates will be sent to the central laboratory for confirmatory identification and antimicrobial susceptibility testing. Aliquots of these frozen bacterial isolates will be stored at a local laboratory for future research up to 5 years after the end of the trial, should participants agree (with the exception of the Netherlands where bacterial isolates will be kept up to 15 years as per standard local practice).
- The MIC, defined as the lowest concentration of an antimicrobial that inhibited the visible growth of a microorganism after overnight incubation, will be determined for the following antibiotics: zoliflodacin, azithromycin, cefixime, ceftriaxone, ciprofloxacin, gentamicin, tetracycline and spectinomycin. Detailed methods for MIC determination can be found in the trial laboratory manual. Confirmatory identification of NG by the central lab will constitute data for the primary outcome of the trial.
- WGS will be conducted on all NG isolates cultured at the TOC and EOT visits, as well as on pre-treatment isolates from the respective participants and on baseline isolates with zoliflodacin MIC >0.25 µg/mL. The main objective is to investigate relationships between pre-treatment and post-treatment NG isolates within those participants whose cultures at TOC are found positive for NG, evaluate the likelihood of re-infection *vs* treatment failure and identify possible genetic determinants of zoliflodacin resistance. The analyses will be performed at the microbiology laboratory of the University of Örebro in Sweden.

7.3. Pharmacokinetic evaluations

The PK sub-study will be run at select sites (see MOP for list).

The plasma concentration of zoliflodacin will be determined in the following subsets of participants:

- HIV negative participants and HIV positive participants (≥ 18 years old)
- HIV negative adolescents (≥12 and <18 years old)

Each group will include approximately 20 to 30 participants, with PK assessed at five time-points:

- 15 min – 1 h post treatment
- 2 h – 2.5 h post treatment
- 4.5 h – 5 h post treatment
- 10 h - 12 h post treatment
- 24 h - 36 h post treatment

The time periods will cover C_{max} and at least a 10-fold decline in plasma concentration from this, this decline being adequately defined as mono-exponential. Sampling time post dose will be accurately recorded in the eCRF. Plasma concentrations of zoliflodacin will be determined by K-CAS laboratories using a validated high-performance liquid chromatography (HPLC) – mass spectroscopy (MS)/MS method.

At each time point, a 4 ml venous blood sample will be collected. Samples will be centrifuged to allow for extraction of plasma. Plasma should be frozen at about – 70 °C within a maximum of 60 minutes after collection of the blood samples. Further details are available in the MOP.

The same food intake recommendations detailed in Section 5.2.1 apply to the participants to the PK sub-study.

7.4. Clinical management of participants

Assessments performed at site level will be used for patient management. This includes HIV and pregnancy testing. Safety laboratory results will be communicated to sites in a timely manner to ensure appropriate management of any laboratory finding that is out-of-normal range (see laboratory manual for details).

Table 4 highlights how microbiology test results should dictate clinical management of participants and sampling schedule in the trial.

Table 4: **Assessments and use for clinical management**

<table>
<tr><th colspan="2" rowspan="2"></th><th colspan="2">Action(s) if result is:</th></tr>
<tr><th>Positive</th><th>Negative</th></tr>
<tr><td rowspan="2">Baseline results[1, 4]</td><td>NG culture and NG NAAT</td><td>If either culture and/or NAAT is/are positive, take further swabs for both culture and NAAT at TOC</td><td>If both NG culture and NG NAAT are negative, no further swabs to be taken for NG in the trial</td></tr>
<tr><td>CT NAAT</td><td>○ Treat as per standard of care at TOC after collection of all specimens if participant was randomised to zoliflodacin group
○ No further swabs to be taken for CT (except if participant is symptomatic at day 30)</td><td>No further swabs to be taken for CT (except if participant is symptomatic at day 30)</td></tr>
<tr><td rowspan="2">TOC results</td><td rowspan="2">NG culture and NG NAAT</td><td>○ If NG culture is positive, treat as per standard of care
○ No further swabs to be taken for NG[2]</td><td>If both NG culture and NG NAAT are negative, no further swabs to be taken for NG in the trial (except if participant is symptomatic at day 30)[1, 3]</td></tr>
<tr><td colspan="2">○ If NG culture is negative and NG NAAT is positive at TOC, both NG culture and NG NAAT to be done at day 30[1]</td></tr>
<tr><td>Day 30 results[3]</td><td>NG culture and NG/CT NAAT</td><td>Treat as per standard of care if any of the test results is positive</td><td></td></tr>
</table>

CT: *Chlamydia trachomatis*; NAAT: nucleic acid amplification test; NG: *Neisseria gonorrhoeae*.
[1] Applies to each of the three anatomical sites independently.
[2] If NG culture is positive in any of the three anatomical sites, no further swabs need be taken from any site at day 30, except if participant is symptomatic at day 30.
[3] If a participant presents with signs and/or symptoms of an NG infection at Day 30, swabs for NG culture and NG/CT NAAT should be collected from all three anatomical sites.
[4] In the event that Baseline results are not available at the time of the TOC visit, all swabs from each of the three anatomical sites should be taken.

Antimicrobial susceptibility testing will be performed in batches during the trial and therefore will not be available to sites for clinical management. WGS is exploratory in nature and will moreover be analysed in batches. As a consequence, results are not to be used for clinical management.

8. Assessment of safety

8.1. Definitions

8.1.1. Adverse event

An AE is defined as any untoward medical occurrence in a clinical trial participant administered a medicinal product and which does not necessarily have a causal relationship with this treatment.

An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding or a procedure abnormality that are deemed clinically significant by the investigator), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

Furthermore, the definition of an AE includes worsening (in severity and frequency) of pre-existing conditions ("medical history") after trial drug administration and abnormalities of procedures (i.e. electrocardiogram, X-ray…) or laboratory results which are assessed as “clinically significant”.

What laboratory/procedures abnormalities should be considered as an AE?

All treatment-emergent laboratory/procedures abnormalities (or worsening in severity or frequency of pre-existing abnormalities) assessed as “clinically significant” must be reported as an AE.

When reporting an abnormal laboratory/procedure result, a clinical diagnosis should be recorded rather than the abnormal value itself, if available (for example, “anaemia” rather than “decreased red blood cell count”).

What is not an AE?

- Medical conditions or abnormal safety laboratory results present at the initial trial visit that do not worsen in severity or frequency during the trial are NOT considered as AE and should be documented as medical history if significant.
- Symptoms, exacerbation or worsening of the studied disease will NOT be considered as AE and will not be captured in the AE page of the eCRF if consistent with the anticipated natural progression of the disease (overall and for this given participant).
- Lack of efficacy of the trial treatment is NOT considered as AE.

Special Cases: screening period:

With the exception of abnormal safety laboratory findings detected at baseline (which are assumed to have been present prior to informed consent), events occurring after informed consent but prior to study treatment administration must be reported as adverse events using the applicable form in the eCRF, but will not be classified as treatment-emergent adverse events.

8.1.2. Adverse Event reporting period

The adverse events reporting period begins upon participant enrolment in the trial (after signature of informed consent) and ends at an EOT or early withdrawal visit.

All adverse events that occur during the adverse event reporting period specified in the protocol must be reported, whether the event is considered medication related or not. In addition, any adverse event that occurs after the adverse event reporting period that the investigator assesses as possibly related to the investigational medication should also be reported as an adverse event.

Special Cases: screening failures:

For screening failure participants, events and updates must be recorded in the eCRF and using the AE or serious adverse event (SAE) forms (as appropriate) until the date the participant was determined to be a screening failure. Beyond that date, only serious or medically relevant protocol-related events will be followed-up. The date and time of screening failure will also be recorded in the source notes and the eCRF.

8.1.3. Serious adverse event

An AE is defined as serious if it:

- results in death
- is life-threatening: in this context refers to an AE in which the participant was at risk of death at the time of the AE; it does not refer to an AE that hypothetically might have caused death if more severe
- requires or prolongs hospitalization, *i.e*. the AE requires at least an overnight admission or prolongs a hospitalisation beyond the expected length of stay. Hospital admissions for social reasons and respite care in the absence of any deterioration in the participant's general condition, as well as hospitalisation for elective surgery or for normal disease management (including treatment adjustment) are NOT to be considered as SAE according to this criterion
- results in persistent or significant disability, i.e. the AE resulted in a substantial disruption of the participant's ability to conduct normal activities
- is a congenital anomaly/birth defect, *i.e*. an AE outcome in a child or foetus of a participant exposed to the IMP before conception or during pregnancy
- is an important medical event.

Medical and scientific judgment should be exercised in deciding whether other situations should be considered serious events, such as important medical events that might not be immediately life-threatening or result in death or hospitalisation but might jeopardise the participant or might require intervention to prevent one of the other outcomes listed above. Examples of such events include, but are not limited to, intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization. In addition, any suspected transmission via a medicinal product of an infectious agent is also considered a serious adverse event/reaction.

Although exposure during pregnancy and overdose are not always serious by regulatory definition, these events must be handled as defined in Section 8.4 for reporting trial treatment exposure during pregnancies and Section 8.1.4 for overdose.

8.1.4. Overdose

An overdose is defined as a dose greater than the dose evaluated in the present trial as described in Sections 6.1 and 6.2 of the protocol.

All overdoses should be reported in the eCRF. If the overdose is associated with an AE, then the AE should also be reported.

In the event of an overdose of trial treatment, the investigator should use clinical judgment in treating the overdose and contact the sponsor medical monitor. The investigator should refer to the IB for detailed information regarding warnings, precautions, contraindications, AEs, and other significant data pertaining to zoliflodacin. Investigators should refer to ceftriaxone and azithromycin SmPCs respectively in case of overdose with either comparator treatment.

Trial treatment dosing errors made by the research facility staff that result in a possible overdose (as well as all dosing errors) of trial treatment must be documented as protocol deviations and reported to the sponsor and the IRB/IEC in accordance with the IRB/IEC requirements.

8.1.5. Grading of AE severity

The severity is used to describe the medical intensity of a specific event. In this clinical trial, the severity of AEs must be primarily assessed by investigators according to the Common Terminology Criteria for Adverse Events (CTCAE) (54) tables as a guide in the grading of the severity of AEs (grades 1-5). The tables will be provided in the MOP.

Whenever the AE grading is not detailed in the CTCAE tables, the investigator should use the following grading for that specific AE:

MILD: The participant is aware of the event or symptom, but the event or symptom is easily tolerated. Does not interfere with the trial participant's activities of daily living (ADL).

MODERATE: The participant experiences sufficient discomfort to interfere with or reduce his or her usual level of ADL.

SEVERE: Significant impairment of functioning: the participant is unable to carry out usual ADL and/or the participant's life is at risk from the event.

LIFE-THREATENING: The participant's life is at significant risk; extreme limitation in ADL, significant assistance required; significant medical intervention/therapy required, hospitalization or hospice care probable.

The investigator should continue using the above grading for all other subsequent evaluations of this particular AE for that participant and all others. When the intensity of an AE changes over time, each change in intensity will be recorded in the source documents until the event resolves. However, only one AE and the maximum intensity will be recorded in the eCRF for each separate event. If the AE resolves but then recurs, each will be recorded as a separate AE, with the appropriate start and stop dates and times.

8.1.6. AE causality assessment

For both serious and non-serious AEs, the investigator is required to assess if there is a causal relationship between the AE and the trial treatments, *ie.* to determine whether there exists a reasonable possibility that the trial treatments caused or contributed to the AE.

The following categories for relationship to each trial treatment will be used during AE reporting:

RELATED: There is at least a reasonable possibility of causal relationship between the AE and the trial treatment. This means that there are facts (evidence) or arguments to suggest a causal relationship.

NOT RELATED: There is no reasonable possibility that the AE is related to the trial treatment.

8.2. Detection and recording of AEs

The investigator is required to report all directly observed adverse events and all adverse events spontaneously reported by the trial participant using concise medical terminology. In addition, at each visit the participant will be questioned about the occurrence of adverse events, with a generic question such as "*Since last visit have you felt any health problem?*". AEs can be spontaneously reported by trial participants or elicited through open-ended questioning, examination, or evaluation of trial participants during trial visits. The investigator should also review all results of assessments performed as part of the trial, such as laboratory assessment results and physical examinations, and assess them for clinically relevant changes compared to baseline (Section 8.1.1).

All AEs occurring during the trial must be recorded in the AE section of the eCRF. AE recording applies to both investigational and control group. AEs (including lab or procedures abnormalities) should be described using a diagnosis whenever possible, rather than individual underlying signs and symptoms. When a clear diagnosis cannot be identified, each sign or symptom should be reported as a separate AE.

As far as possible, each AE should be evaluated to determine:

- The severity grade based on the CTCAE (54) Grade 1-5 (Section 8.1.5) **or** on grading scale described above (only for those AEs not described in CTCAE)
- Its duration (start and end dates or if continuing at the end-of-trial visit)
- Its relationship to each trial treatment (causality; see Section 8.1.6)
- Action taken with respect to each trial treatment (treatment maintained, dose reduced, permanently discontinued, temporarily discontinued, not applicable)
- Whether medication or therapy was taken/given in relation to the AE
- Whether it is serious (see Section 8.1.3).

AE recording shall begin upon signature of the informed consent form (ICF) and should continue until the participant's last visit.

8.3. Expedited reporting of SAEs

8.3.1. Reporting of SAEs to sponsor

Every SAE occurring in a trial participant during the trial (whether or not the event is considered trial treatment related) must be reported by the investigator to the sponsor on SAE e-mail address (mentioned below) within 24 hours of learning of its occurrence, preferably using an SAE report form in English (dated, signed and scanned). All relevant information concerning the SAE must be obtained from the appropriate points of contact, such as description of the event, onset date and type, duration, severity, relationship to each trial treatment, outcome, actions taken, emergency room records, laboratory testing and

treatment of the SAE, and all other relevant clinical and laboratory data as soon as possible.

SAE email address: SAESTI_Zoli001@gardp.org

The original copy of the SAE report form and the confirmation of receipt email must be kept with the participant medical file at the trial site.

Recurrent episodes, complications, or progression of the initial SAE must be reported as follow-up to the original episode within 24 hours of the investigator receiving the follow-up information.

Alike non-serious AEs, information about all SAEs should also be collected and recorded in the AE section of the eCRF.

8.3.2. Reporting of SAEs to national regulatory authorities and ethics committees

Related SAEs and SUSARs will be reported in an expedited manner to the national regulatory authorities (NRA), ethics committees and any other relevant institutions/bodies as per local safety reporting regulations. Further details will be provided in the trial safety management plan.

8.4. Exposure during pregnancies

The effect of zoliflodacin administration has not been studied in pregnant women. Therefore, all eligible women of reproductive age must not be pregnant and have a negative pregnancy test before they can be included in the trial. All female participants must also agree on using a highly effective method of contraception at the time of IMP administration on Day 1 (see Appendix 1 for acceptable methods and required duration) and until at least 28 days after administration of the trial treatment.

All participants must be instructed to inform the investigator immediately if there is a suspicion of pregnancy. If following trial treatment, it is subsequently discovered that a trial participant, or the female partner of a trial participant, is pregnant or may have been pregnant at the time of investigational drug exposure, the sponsor must be notified immediately (within 24 hours). The principal investigator must report all cases of exposure to study treatment during pregnancies using the “pregnancy surveillance form”. This must be done irrespective of whether an adverse event has occurred. The information submitted should include the anticipated date of delivery.

The investigator must follow the women exposed during pregnancy from day of awareness of pregnancy until completion of the pregnancy at definite intervals (at 3 months, 6 months, at 9 month/child birth) or until pregnancy termination (i.e., induced / spontaneous abortion). The investigator will provide pregnancy outcome information as a follow up to the initial pregnancy surveillance form.

Stillbirth infants should be seen by a doctor as soon as possible to assess the cause of death. In the case of a live birth, a doctor (preferably a paediatrician if possible) should assess the infant at the time of birth and submit a child surveillance report to the sponsor.

Follow-up of children exposed to drug in utero is proposed to the parents by the Investigator, until the children reach 2 years of age. If an event presented by a child exposed to drug in utero during child monitoring is identified as meeting a seriousness criterion (e.g. congenital anomaly or death), it shall be reported using the SAE form (in addition to the “Child Surveillance form”) within the same timelines as for SAE reporting (see timelines in Section

8.1.2).

8.5. AE follow up

All AEs should be treated appropriately by the investigator. Once an AE is detected, it should be followed until its resolution or until stabilization. Assessment should be made at each visit (or more frequently, if necessary) of any changes in severity, suspected relationship to the trial regimen, interventions required to treat it, and outcome. For AEs that have not resolved at the end of the trial, it is the responsibility of the principal investigator to ensure that participants are appropriately referred to their treating physician or to an adequate specialist.

In addition, all serious adverse events and those non-serious events assessed by the investigator as related to the investigational drug must continue to be followed even after the subject participation in the trial is over. Such events should be followed until they resolve or until the investigator assesses them as “chronic” or “stable”.

8.6. Safety assessments

Safety assessments include physical examination, safety lab assessment and review of potential AEs. They will be performed at each visit, as per the schedule of events (Section 5).

9. Withdrawals

At any point in time, participants may withdraw consent. Participants who withdraw from the trial will benefit from the same conditions of care as those who remain in the trial. Samples collected up until the date of withdrawal will be kept and analyzed, and this will be explained in the ICF.

The investigator is responsible for completing a final assessment form in the eCRF at the time of the participant’s withdrawal. The investigator should make a reasonable effort to ascertain the reason(s) for consent withdrawal, while fully respecting the participant's rights.

If a participant does not return for a scheduled visit, every effort should be made to contact him/her. In any circumstance, every effort (a minimum of 2 phone calls, documented in the source documents) should be made to document participant outcome, if possible.

The investigator may also decide to withdraw a participant from the trial at any time without penalty and for any reason without prejudice to his or her future medical care. Participants may be withdrawn for non-compliance with trial procedures or trial drug administration.

In all cases, the date and reason(s) for withdrawal (including the primary reason) must be recorded in the source documents and the eCRF. If a participant is prematurely withdrawn from the trial for any reason, the investigator must make every effort to perform the evaluations in accordance with Section 5.2.6. Every effort will be made to follow up participants who prematurely discontinue from the trial to determine final outcome.

10. Data collection and management

10.1. Source data

According to the International Council on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), source documents are “original documents, data and records (*e.g.* hospital records, clinical and office charts, laboratory notes, memoranda, participant’s diaries or checklists, pharmacy dispensing records, recorded data

from automated instruments, microfiches, photographic negatives, microfilms or magnetic media, X-rays, participant files, and records kept at the pharmacy at the laboratory and at medico-technical departments involved in the clinical trial)" (ICH E6, 1.52)

According to the ICH, source data are "all information in original records and certified copies of original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents (original records or certified copies)" (ICH E6, 1.51).

In the present trial, all trial data will be first recorded in source documents before being transcribed in the eCRF or data provided to PV.

The source documents must remain available at the trial site after data have been transcribed in the eCRF/data or forms sent to PV, to allow retrospective checks that source data have been accurately and completely transcribed in the eCRF/data sent to PV. The investigator will allow the monitors, the persons responsible for the audit, the representatives of the ethics committees, and of the regulatory authorities to have direct access to source data and documents in order to analyse, examine, verify and reproduce any report and record that is important for the evaluation of the trial.

10.2. Electronic data capture system

An eCRF will be designed to record all the data to be collected as per the protocol. An eCRF will be completed for each participant. The eCRF, together with all trial related forms and logs will be produced by the sponsor before being distributed to investigators. The eCRFs have been built using Clinical Studio, a fully validated secure web-enabled software that conforms to 21 CFR Part 11 requirements.

The designated investigator staff will enter the data required by the protocol into the eCRF. The principal investigator will be responsible for assuring that the data entered into the eCRF is complete, accurate, consistent with the source documents and that entry and updates are performed in a timely manner (including in consistency with data sent to PV). Corrections and alterations of data on the eCRF or source documents must be made by the investigator or by the designated person from his/her team and must be dated and signed. Investigator site staff will not be given access to the electronic data capture (EDC) system until they have been adequately trained.

Medical history/current medical conditions and adverse events will be coded using the MedDRA terminology. Prior and concomitant medications will be coded using WHO-DRUG dictionary.

ICON GPHS data management will review the eCRF data entered by investigator staff for completeness and accuracy. Electronic data queries stating the nature of the problem and requesting clarification will be created for discrepancies and missing values and sent to the investigational site via the EDC system. Designated investigator site staff are required to respond promptly to queries and to make any necessary changes to the data.

Protocol deviations will be detected through appropriate monitoring (see Section 13 quality assurance and quality control). These should be appropriately documented and upon conclusion of the trial, the list of deviations will be communicated to ICON GPHS data management and statistics. Once the trial data has been verified for completeness and accuracy, the database will be locked.

11. Data analysis and statistical methods

11.1. Sample size determination

The efficacy of the combination therapy 500 mg IM ceftriaxone and 1 g oral azithromycin in the SOLITAIRE-U trial was initially used as the cure rate reference (55, 56). This trial had 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 (56). In addition, the trial observed 14% of participants with indeterminate outcome (participants who did not return for TOC assessment).

For this study, the initial assumption was that there would be no treatment difference between the parenteral comparator ceftriaxone and azithromycin) and zoliflodacin. A 10% non-inferiority margin was initially selected, based on the US FDA guidelines on drug development for NG (38). An unequal allocation ratio was chosen to provide sufficient data on the safety of zoliflodacin (57). The initial hypothesis was that a single oral 3 g of zoliflodacin would 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 was 10% or less (prespecified non-inferiority margin for the primary endpoint). Based on an 85% cure rate in the comparator arm (combination therapy), and a non-inferiority margin of 10%, it was calculated that 603 participants (402 in the intervention group and 201 in the control group, with a two-sided α of 5% and 2:1 allocation ratio) would provide 90% power to show that a single oral 3 g dose of zoliflodacin was 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 11.2.1), it was estimated that a maximum of 35% of the randomized population would be potentially ineligible for inclusion in the micro-ITT population. Therefore, it was anticipated that up to approximately 928 participants would be randomized into the trial in order to achieve the target sample size of 603 in the micro-ITT population. Accounting for anticipated screening failures, the enrolment sample size was estimated to be around 1092.

During the study however, GARDP reassessed the cure rates of all recent Phase 2 and 3 trials investigating the efficacy of a new oral investigational product for uncomplicated gonorrhoea (24, 26, 56, 58). 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. This is hypothesized to be due to intrinsic differences in the bioavailability of an oral versus parenteral drug. Under these assumptions, the oral therapy would still be estimated to yield a true cure rate of over 95% which is acceptable for single dose oral therapy for gonorrhoea as stated in the CDC criteria (59, 60).

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 (61). The analysis proposed that by reviewing data from more recent studies, a non-inferiority 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.

Therefore in a Type C interaction with the US FDA held in October 2022 wherein GARDP requested consideration by the Agency of an increase in the non-inferiority margin, taking into account the -4% treatment difference, the published meta-analysis (61) and the unmet public health need for new drugs active against ceftriaxone-resistant NG (see Section 1.3), the US FDA indicated that a wider non-inferiority margin of 12% for the primary endpoint could be acceptable. In the Appendix 'Justification for noninferiority margin', the US FDA guidance (38) estimates the benefit of an effective treatment over placebo to be 11.9%, but this is acknowledged by the US FDA to be a conservative estimate due to the limitation of the data included in the meta-analysis. Additionally, the Agency added that, dependent on demonstrated efficacy, consideration would be given for a broad or limited population indication for the treatment of uncomplicated gonorrhoea.

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 12% or less (prespecified non-inferiority margin for the primary endpoint).

Keeping in mind the trial was conducted for the most part during the high peaks of the COVID-19 pandemic, monitoring of blinded pooled data was conducted to assess the performance of the study against assumptions used in the sample size determination described above in these higher risk circumstances. When approximately 70% of participants were enrolled, the proportion of participants ineligible for the micro-ITT population were estimated to be approximately 20% (vs 35% in original assumptions), and the proportion of nonassessable outcomes due to missed TOC assessments at 10% (foreseen at 15% in original assumptions).

Based on these estimations, a blinded pooled data analysis was performed taking into account as well the-4% treatment difference and a 12% non-inferiority margin. It was concluded 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 11.2.1) and blinded analysis 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 (remains unchanged). Similarly, accounting for anticipated screening failures, the enrolment sample size remains estimated to be around 1092.

11.2. Definition of trial populations included in the analysis

For each microbiological outcome and anatomical site (see Section 2), 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.

11.2.1. Micro-ITT population

The micro-ITT population will include all randomized participants who had a positive NG culture at baseline and whose baseline antimicrobial susceptibility testing result showed no pre-existing resistance to both ceftriaxone and azithromycin. 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 will be defined as per local standards. Further details may be found in the SAP.

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 modified micro-ITT population will include all randomized participants who had a positive NG culture at baseline.

11.2.2. Clinical cure population

For the efficacy analyses of clinical cure, 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.

11.2.3. Evaluable population

The evaluable population 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.

11.2.4. PP population

The PP efficacy population 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 and 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).

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

11.2.5. Safety population

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

11.2.6. PK population

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

11.3. Baseline characteristics

Baseline demographics and clinical characteristics will be described overall and by treatment group and trial site. Demographic data will include age, gender, sex at birth and ethnicity. Baseline clinical data will include initial clinical presentation, sexual history, previous or coexisting sexually transmitted infections, results of laboratory investigations including results of culture, NAAT and Gram stain, and antibiotics susceptibility testing where available. Summary tables will present descriptive statistics: for continuous variables, mean ± standard deviation or median and range (whichever is most appropriate); for categorical variables, frequency counts and percentage of participants within each category.

Any missing data pattern will be investigated and reasons for missing data obtained and summarised where possible. A second table summarising baseline characteristics of participants by trial 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.

11.4. Efficacy analysis

11.4.1. Definitions

- Microbiological cure: a participant with a positive NG culture at baseline and a negative NG culture at the TOC visit. A positive NG culture is defined as a culture with confirmed microbiological identification by the central laboratory.
- Microbiological failure: a participant with a positive NG culture at baseline and at the TOC visit and for whom there is no supportive evidence of a re-infection or a participant experiencing an intercurrent event before the TOC visit which is possibly related to a lack of efficacy of the study treatment. Supportive evidence of re-infection includes re-exposure to an untreated sex partner or a NG strain genotype different from the strain identified at baseline and/or history of unprotected sex. 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.
- Clinical cure: a male at birth participant experiencing resolution of signs and symptoms of urogenital gonococcal infection that were present at enrolment. Further details on assessment of clinical cure are included in the MOP.
- Lost-to-follow-up: 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± 3 days)
- Non-evaluable for microbiological outcome: a participant who did not have a urogenital positive culture result for NG at baseline (visit 1).

11.4.2. Specification of efficacy analysis

Efficacy endpoints are defined in Section 2. The primary efficacy endpoint is defined as a negative culture to NG at urethral or cervical sites at TOC (day 6 ±2) among patients included

in the micro-ITT population.

The difference in the proportions of participants, in the micro-ITT population, with negative culture at the TOC visit will be estimated between the ceftriaxone/azithromycin and the zoliflodacin groups and a 2-sided 95% CI will be calculated. 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 lies below the 12% prespecified non-inferiority margin. The primary analysis will be conducted on the micro-ITT analysis, which includes participants who are non-assessable for microbiological outcome or who are lost-to-follow-up.

To include all randomized participants, missing outcomes will be replaced using multiple imputation (62). Sensitivity analyses will also be performed to explore departures from assumptions about missing data. To take into account the possibility that missing outcomes are not completely at random, a pattern mixture model will be fitted under missing at random (MAR) and it will be expanded to allow for the context of missing not at random (MNAR) (62). 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 (62). This efficacy analysis will also be performed on the PP and evaluable population as part of the secondary objectives.

For secondary efficacy endpoints, Chi-2 or Fisher exact tests will be performed, depending on applicability criteria, for the comparison of:

1) The proportions of microbiological cure at TOC between both trial groups at pharyngeal and rectal sites
2) The proportions of clinical cure at TOC between both trial groups.

These analyses will be conducted in the micro-ITT population and repeated in the modified micro-ITT, the evaluable and PP populations, unless stated otherwise. In particular, the proportion of clinical cure will be assessed in the clinical cure population. All statistical tests will be 2-sided with a level of significance set at 5%. In addition, the following comparison will be made

3) The proportions of participants with a negative NG NAAT from urethral or cervical sites at TOC between both trial groups
4) The proportions of participants with a negative NG NAAT from oropharyngeal sites at TOC between both trial groups
5) The proportions of participants with a negative NG NAAT from rectal sites at TOC between both trial groups

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

Regarding the antimicrobial susceptibility testing profile of gonococcal isolates, all analyses will be descriptive. The MIC breakpoint plus the median, 90^{th} percentile, 50^{th} percentile, modal MIC and range of MICs will be reported by treatment group and by site of infection. 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 antimicrobial susceptibility testing between the baseline and TOC isolates will be reported for

all participants with microbiological failure without any statistical comparison.

A descriptive analysis of participants with baseline isolates resistant to ceftriaxone, to azithromycin and to both will also be reported. Sensitivity analyses will also be performed.

In addition, the following exploratory, comparative analyses will be conducted using Mann-Whitney non-parametric test:

- Antimicrobial susceptibility testing profile (MIC distribution) of baseline and TOC isolates for participants with multiple sites of infection vs participants with unique site of infection.
- Antimicrobial susceptibility testing profile (MIC distribution) of baseline and TOC isolates for isolates that are resistant to fluoroquinolones at baseline vs isolates that are fluoroquinolone-sensitive.
- Antimicrobial susceptibility testing 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) at baseline vs isolates that are sensitive to those antibiotics.
- Antimicrobial susceptibility testing profile (MIC distribution) of baseline isolates of participants with subsequent microbiological failure vs microbiological cure.

11.5. Safety analysis

Safety endpoints are defined in Section 2. All safety analyses will be descriptive, and endpoints will be reported by trial group.

AEs will be coded using the latest version of the MedDRA. All AEs and SAEs occurring after trial drug dosing will be summarized using frequency counts and percentages and their exact 95% CI in the following groups:

- Overall (*i.e.*, regardless of severity or relationship to treatment);
- By severity grade (mild, moderate, or severe);
- By relationship to trial drug;
- By MedDRA level hierarchy (system organ class [SOC], higher level group term [HLGT], and preferred term [PT]).

Descriptive statistics (mean, standard deviation, 95% CI or median and interquartile range, whichever is appropriate) 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 and those that are clinically significant will be highlighted and reported as AE.

11.6. Pharmacological endpoints

The following PK parameters will be estimated for zoliflodacin:

- $AUC_{(0\text{-last})}$: Area under the concentration time-curve from time zero to the last concentration above the lower limit of quantitation
- $AUC_{(0\text{-}\infty)}$: Area under the concentration time-curve from time zero to infinity
- $AUC_{(0\text{-t})}$: Area under the concentration time-curve from time zero to time t
- C_{max}: Maximum observed concentration
- T_{max}: Time of maximum observed concentration
- K_e: Elimination rate constant
- $t_{1/2}$: Terminal elimination half-life

- CL/F: Apparent total clearance of the drug after oral administration
- Vz/F: Apparent volume of distribution

The PK analysis will be addressed in a separate report.

11.7. Missing values

Prior to analysis, any missing data will be investigated and reasons for missing data obtained and summarised where possible. All attempts will be made to avoid any missing outcome data and to correct for it. Because the definition of the micro-ITT includes participants who are non-assessable for microbiological outcome or who are lost-to-follow-up, we will replace missing outcomes using multiple imputation (as described under 11.4). 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). 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 (62). 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 (62). For those participants with a missing primary outcome from the central laboratory but with existing outcome from the local microbiology laboratory, the local laboratory data will be used, and a sensitivity analysis will be performed. A sensitivity analysis measuring the impact of the different reasons for missing values will also be performed.

12. Data safety monitoring board

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.

13. Quality assurance and quality control procedures

13.1. Monitoring, audits and inspections

Prior to trial start, a monitoring plan will be developed. The site principal investigator will allow the monitors to visit the site and facilities where the trial will take place in order to verify compliance with the trial protocol, ICH-good clinical practice (GCP) and WHO-good clinical laboratory practice (GCLP) for laboratories. Training sessions on GCP, GCLP and on protocol implementation will be organised for the investigators and all trial staff prior to recruitment start. A MOP will be distributed to all the trial centres and a laboratory manual will be distributed to all laboratories.

Trial monitoring will be carried out by dedicated monitors at ICON GPHS at regular intervals, depending on the recruitment rate, to verify data quality and trial integrity. When travel of monitors and/or access to clinics is limited due to COVID-19 restrictions, remote monitoring is allowed to ensure continuity of monitoring where permitted and feasible locally.

The principal investigator must allow the monitor to:

- inspect the site, the laboratories, the facilities, the equipment and the material used for the trial,
- meet all members of his/her team involved in the trial,
- consult all of the documents relevant to the trial, including those filled by the trial nurse and trial pharmacist
- check that the eCRF has been correctly completed
- review the completion and accuracy of pharmacovigilance documentation and consistency with the eCRF
- directly access source documents for comparison of data therein with the data in the eCRF/data or forms sent to PV,
- verifying that randomization has been conducted in accordance with the corresponding SOP and that no breach occurred in allocation concealment
- verify the collection, transport, storage and shipment of biological samples
- verify that the trial is carried out in compliance with the protocol and national regulatory requirements
- verify the proper handling and management of trial treatments

At the end of each monitoring visit, and based on monitoring visit reports, the sponsor will be responsible for controlling:

- recruitment rates, ineligibility, non-compliance, protocol violations and dropouts overall and in each trial centre
- completeness and timeliness of data
- compliance with GCP, GCLP and applicable regulations
- any protocol deviations as defined in the monitoring plan.

A final monitoring visit will be conducted at the end of the trial, after the last participant last

visit (LPLV), and once the database is locked.

13.2. Audits and inspections

In addition to the monitoring activities foreseen above, the trial may be evaluated by external auditors appointed by the sponsor and by representatives from national regulatory authorities or ethics committees who must be allowed access to eCRFs, source documents, trial files, and trial facilities.

13.3. Laboratory framework

All laboratory activities will be carried out according to the trial laboratory manual. The laboratory manual compiles all procedures that are relevant to the evaluation of trial outcomes, and encompass pre-analytical, analytical and post-analytical phases.

Most laboratory investigations to be performed during the trial are already in use in the trial sites. Hence existing quality control (QC) procedures will be followed for all laboratory investigations. Additional quality assurance activities will be implemented as necessary to meet GCLP standards, and oversight will be provided by the sponsor. Details can be found in the trial laboratory manual.

14. Trial documentation

14.1. Confidentiality and retention of trial documents

Due to the social harm and stigma that is often associated with STIs, all trial sites are accustomed to maintaining strict confidentiality with regards to their patients. Most sites are dedicated STI clinics. All trial-related information will be stored securely at the trial sites. All participant information will be stored in locked file cabinets in areas with access limited to site staff.

All laboratory specimens, reports, trial data collection, process, and administrative forms will be identified by a coded number only to maintain participant confidentiality. All records that contain names or other personal identifiers, such as locator forms and informed consent forms, will be stored separately from trial records identified by code number. All local databases will be secured with password-protected access systems. Forms, lists, logbooks, appointment books, and any other listings that link participant ID numbers to other identifying information will be stored in a separate, locked file in an area with limited access.

Participant’s trial information will not be released without the written permission of the participant, except as necessary for monitoring by the sponsor, the DSMB, the NRAs or IRBs/IECs. The principal investigator should agree to provide access to all trial information and documents to sponsor representatives, auditors and inspector mandated by NRAs. The information will be treated in compliance with professional secrecy.

The investigators must arrange for the retention of all information relevant to the trial, including a copy of the eCRF along with audit trail and essential documents listed in Section 8 of ICH-GCP, for at least 15 years after completion or termination of the trial. After that period of time, the documents may be destroyed with prior permission from the sponsor, subject to local regulations. Should the investigator wish to assign the trial records to another party or move them to another location, the sponsor must be notified in advance.

14.2. Protocol amendment

If the protocol must be altered after it has been signed, the modification or amendment must

be discussed and approved by the sponsor. The protocol amendment must be drafted and signed by both parties. All amendments must be submitted to the relevant ethics committees and NRAs. Administrative amendments can be implemented immediately but amendments that affect other aspects can only be implemented after a favourable opinion of the ethics committee and NRA has been obtained and local regulatory requirements have been complied with. An amendment needed to eliminate immediate hazards to the participants in the trial is exempted from this rule. When the submission is performed by the principal investigator, the latter must transmit a copy of ethics committee's new written opinion to the sponsor, immediately upon receipt.

When a protocol amendment results in revised informed consent forms, after IRB/EC approval, re-consent will be obtained from participants currently enrolled into the trial in a timely manner at or before the participant's next trial visit. This will allow sufficient time and information to make a decision about continued participation in the research trial.

14.3. Final trial reports

A final trial report summarising the trial outcome will be drafted by the investigators and provided to the local IEC/IRB.

A clinical trial report will be prepared by the sponsor with input from investigators as appropriate. One copy of the final trial report must be dated and signed by the sponsor's medical monitor, principal investigators, trial statistician and the clinical trial manager before being transmitted to the IRB/IECs and NRAs.

15. Early termination of the trial

Both the sponsor and the investigators reserve the right to terminate the trial at any time prior to inclusion of the intended number of participants, but they intend to exercise this right only for valid scientific or administrative reasons. Should this be necessary, both parties will arrange the procedures after review and consultation. In terminating the trial, the sponsor and the investigators will ensure that adequate consideration is given to the protection of the participants' interests.

Reasons for early termination of the trial by the sponsor may include but are not limited to:

- enrolment rate too low
- protocol violations
- inaccurate or incomplete data
- unsafe or unethical practices
- questionable safety of the test article
- following the recommendation of the DSMB or IEC
- administrative decision

Reasons for early termination of the trial by the investigators may be:

- insufficient time or resource to conduct the trial
- lack of eligible participants.

In the event that the trial is terminated early, the investigator has to:

- complete the eCRF to the greater extent possible

- answer all questions of the sponsors or their representatives related to data of participants enrolled at the site prior to trial termination
- ensure that participants enrolled in the trial who had not yet reached a follow up time point are informed promptly and followed up with the necessary medical care
- provide in writing the reasons for their decision to the IEC/IRB and the sponsor
- follow end of trial procedures as per the protocol and MOP.

16. Ethics

16.1. Trial conduct

The trial will be conducted in accordance with the ethical principles laid down in the declaration of Helsinki 1964, as revised in Fortaleza in 2013, the ICH-GCP, the WHO-GCLP and with all applicable national regulations.

16.2. Regulatory and ethical approval

The trial protocol, the participant ICF, the eCRF, up-to-date versions of the IB or SmPC, as well as principal investigators qualifications will be submitted to appropriate IRBs/IECs.

The trial will not start in any centre before written approval by these IRBs/IECs has been obtained, the local regulatory requirements have been complied with, and the signature of the clinical trial protocol of each contractual party involved has been obtained.

16.3. Informed consent process

An ICF in clear, simple language complying with new Common Rule (45 CFR part 46 subpart A) requirements will be provided to the participant. The investigator or designee is to collect written consent from each participant before any trial-specific procedure is conducted. Two original ICFs must be completed, dated and signed personally by the participant and by the investigator or designated trial staff. The participant should be given one signed original form; the second original should be kept by the investigator.

Adolescent participants (≥ 12 and < 18 years old) will be consented according to the practice in use in their country of origin. If required by local research ethics guidelines, assent will be sought from participants and consent will be sought from a legal representative in addition. In countries that recognize the status of emancipated minors, consent will be sought from the participant directly.

If the participant is unable to read, a relative or an impartial witness should be present during the informed consent discussion. The participant must give consent orally and, if capable of doing so, complete, sign (or thumbprint) and personally date the information and consent form. The witness must then complete, sign and date the form to testify of the participant's understanding of the trial information and his/her willingness to participate, together with the investigator.

The ICF will be provided to the principal investigators by the sponsor. Any changes to the ICF suggested by the principal investigator must be agreed to by the sponsor before submission to the IRB/IEC, and a copy of the approved version must be provided to the sponsor after IRB/IEC approval. Any change to the ICF after the initial approval constitutes an amendment and must be submitted for approval to the IRB/IEC, and to the NRA.

16.4. Justification for selection of trial population

The inclusion of minors in the present trial is justified from an ethical perspective by the fact that prevalence of STIs among adolescents is usually high and zoliflodacin given as a single dose appears to be safe based on non-clinical and clinical studies (see IB). This vulnerable group will benefit from the knowledge and intervention generated by the present trial. Gathering of PK data from adolescents, who usually have lower body weights, is key to inform dosing adjustment if necessary.

HIV positive participants will be eligible for inclusion in the trial, as long as their ART does not contain any of the prohibited medications.

16.5. Community engagement

Prior to trial start, relevant communities, such as women's group, youth, men who have sex with men (MSM) groups, and other key populations in trial sites will be informed. Community meetings will be held to inform these groups of the trial prior to the trial. Feedback on the trial concept will also be gathered through community meetings wherever possible. Follow-up meetings will also be done to provide progress of the trial. Moreover, results of the trial will be disseminated to these communities. During these meeting the communities will be assured of maintaining confidentiality and reducing stigma.

16.6. Risks and benefits

Clinical findings with zoliflodacin:

To date, zoliflodacin as a single dose has been well tolerated by both healthy subjects and patients included in the clinical development program. Most commonly reported AEs included excluding infection-related AEs were mild gastro-intestinal events (nausea and diarrhoea) and headache. There were no AEs reported at a frequency of ≥5%

Non-clinical findings with zoliflodacin:

Mild testicular degenerations were observed in rat and dog studies, and reprotoxicity studies in rodent showed a slight adverse effect on male and female fertility as well as embryofoetal development after multiple dosing for 14-28 days. Zoliflodacin has not been studied in pregnant women, and hence all women participants of child bearing potential should not be pregnant or have a negative pregnancy test at enrolment and use effective contraceptive methods for 30 days after receipt of zoliflodacin. Male participants with female partners of child bearing potential must agree to delay conception for 28 days after treatment, and conduct abstinence/use effective barrier contraceptive methods to avoid exposure during pregnancy for 30 days after receipt of zoliflodacin. As with all antibiotics, allergic and hypersensitivity reactions are a potential risk. Please refer to section 6.1.2 of this protocol and to the latest version of the IB for a full description of the identified and potential risks associated with zoliflodacin.

Potential risks associated with ceftriaxone:

The main risk associated with ceftriaxone is the risk of anaphylaxis, and ceftriaxone is contra-indicated in individuals with known severe allergy to cephalosporins or penicillin. In clinical trials, the most frequently reported AEs for ceftriaxone are eosinophilia, leukopenia, thrombocytopenia, diarrhoea, rash, and hepatic enzymes increased. Injection site reaction at site of intramuscular injection are expected and the use of concomitant lidocaine will mitigate risk of severe injection site pain. Please refer to the ceftriaxone SmPC for a full description of

the identified and potential risks associated with ceftriaxone.

Potential risks associated with azithromycin:

As with erythromycin and other macrolides, rare serious allergic reactions including angioneurotic oedema and anaphylaxis (rarely fatal), dermatologic reactions including acute generalised exanthematous pustulosis, Stevens Johnson syndrome, toxic epidermal necrolysis (rarely fatal) and drug reaction with eosinophilia and systemic symptoms have been reported. If an allergic reaction occurs, appropriate therapy should be instituted. Physicians should be aware that reappearance of the allergic symptoms may occur when symptomatic therapy is discontinued. Please refer to the azithromycin SmPC for a full description of the identified and potential risks associated with azithromycin.

Potential risks associated with lidocaine:

Lidocaine is a local anaesthetic of the amide type that is approved for use as a diluent for ceftriaxone. Reactions to lidocaine include cutaneous lesions, urticaria, oedema or anaphylactoid reactions. Pruritis, burning, oedema, erythema, purpura, and bleeding may occur at the local injection site. Allergic reactions may occur as a result of sensitivity to local anaesthetic agents or to the methylparaben used as a preservative in multiple dose vials. Allergic reactions as a result of sensitivity to lidocaine are extremely rare.

Risks associated with trial participation:

Trial procedures related risks include risks associated with some of the trial procedures. For instance, venepuncture causes transient discomfort and may cause fainting. Bruising at the site of venepuncture may occur. Infection at the site is possible but highly unlikely as aseptic technique will be used.

It is possible that participants' involvement in the trial could become known to others, and that a social impact may result. For example, participants could be treated unfairly, or could have problems being accepted by their families and/or communities. In the event that a participant reports a social impact, every effort will be made by trial staff to provide appropriate care and counselling to the participant as necessary, and/or referral to appropriate resources for the safety of the participant.

Benefits from trial participation:

Participants in this trial will benefit from having a comprehensive STI case management including laboratory tests for NG and CT resulting in faster and better-informed treatment of STIs.

If positive, the trial will benefit future patients with gonorrhoea by providing the necessary evidence to support introduction of a new antibiotic against drug sensitive and drug-resistant gonorrhoea. Given the pace at which resistance to ESC is emerging and spreading globally, it is likely that the incidence of treatment failures to the currently-recommended treatment will increase in the near future. At the same time the 5% resistance threshold set by WHO, US-CDC and other organizations will soon be reached, and alternative treatment options are urgently required.

16.7. HIV status and voluntary counselling and testing

HIV positive participants are eligible for inclusion in this trial. Participants without a documented positive HIV diagnosis or documented prescription of ART will undergo voluntary

counselling and testing (VCT) for HIV as part of the trial baseline assessments, and as per local routine procedures. All HIV positive individuals will be offered counselling, referred for HIV care and treatment, in line with routine practice in trial sites. This may either be done at the same time as consent is obtained for inclusion in the trial or at a later date according to hospital practice. Participants who are found to be HIV positive will be referred onwards for treatment, surveillance and follow up according to the national protocol for HIV positive participants. Participants on pre- or post- exposure prophylaxis for HIV infection may enrol in the trial provided they meet all inclusion criteria and none of the exclusion criteria including those pertinent to disallowed concomitant medications.

16.8. Compensation

All trial treatments and diagnostic test costs will be supported by the sponsor. Participants will receive compensation for their time spent for trial participation as per local standards.

16.9. Insurance and liability

As sponsor of the trial, GARDP is liable for and shall indemnify any claims and damages brought by a trial participant suffering from an injury, which arises out of the performance of the trial in accordance with the protocol. To this end, GARDP has taken an insurance covering the conduct of the trial and all participants.

16.10. Post-trial access

If the present trial is successful in demonstrating zoliflodacin is non-inferior to the comparator regimen, the sponsor intends to use trial results to support a request for registration in all trial countries. In addition, the sponsor will develop a stewardship and access strategy that will accompany the roll out of zoliflodacin in target countries and ensure zoliflodacin is promptly available to future gonorrhoea patients.

17. Reporting and publication

17.1. International registry of clinical trials

The present trial was registered in the clinicaltrials.gov public register prior to start (NCT # NCT03959527).

17.2. Publication

Upon trial completion and finalisation of the trial report, the results of the trial will be either submitted for publication and/or posted in a publicly accessible database of clinical trial results. Authorship of any publication will be based on the uniform requirements for manuscripts submitted to biomedical journals as defined by the International Committee of Medical Journal Editors (ICMJE).

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Appendix 1: Contraception methods considered as highly effective

For the purpose of this trial methods that can achieve a failure rate of less than 1% per year when used consistently and correctly are considered as highly effective birth control methods. Such methods, and the duration of required adherence prior to IMP administration, include:

- combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation:
  - oral (required for at least 7 days prior to IMP administration)
  - intravaginal (required for at least 7 days prior to IMP administration)
  - transdermal (required for at least 7 days prior to IMP administration)
- progestogen-only hormonal contraception associated with inhibition of ovulation:
  - oral (required for at least 7 days prior to IMP administration)
  - injectable (required for at least 7 days prior to IMP administration)
  - implantable (required for at least 7 days prior to IMP administration)
- intrauterine device (IUD) (any time prior to IMP administration)
- intrauterine hormone-releasing system (IUS) (any time prior to IMP administration)
- bilateral tubal occlusion (any time prior to IMP administration)
- vasectomised partner[1] (at least 3 months prior to IMP administration and verified by partner semen analysis)

All contraceptive methods must be continued until at least 28 days post IMP administration.

[1] Vasectomised partner is a highly effective birth control method provided that partner is the sole sexual partner of the female of child-bearing potential trial participant and that the vasectomised partner has received medical assessment of the surgical success.