A Phase 1 Clinical Trial to Evaluate the Plasma Pharmacokinetics, Safety, and Tolerability of a Single Oral Dose of Zoliflodacin in Healthy Male and Female Subjects

DMID Protocol Number: 16-0118

DMID Funding Mechanism: Phase 1 Clinical Trial Unit

Pharmaceutical Support Provided by: Entasis Therapeutics

IND Sponsor: Division of Microbiology and Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health

Site Principal Investigator: Barbara Lomeli, MD

DMID Clinical Project Manager: Meg Gordon, RN

DMID Medical Officer: Rick Fairhurst, MD, PhD

DMID Medical Monitor: Soju Chang, MD

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STATEMENT OF COMPLIANCE

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

- United States Code of Federal Regulations (CFR) applicable to clinical trials (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 54, 21 CFR Part 56, and 21 CFR Part 312);
- International Conference on Harmonization (ICH) E6; 62 Federal Register 25692 (1997);
- National Institutes of Health (NIH) Clinical Terms of Award, as applicable.

Compliance with these standards provides public assurance that the rights, safety, and well-being of study subjects are protected, consistent with the principles that have their origin in the Declaration of Helsinki.

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

SIGNATURE PAGE

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

Site Investigator: _____
Name/Title (Print)

Signature: _____

Date: _____

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LIST OF ABBREVIATIONS

AE	Adverse Event
ALT	Alanine aminotransferase
AP	Alkaline phosphatase
AST	Aspartate aminotransferase
AUC	Area Under the Curve
BMI	Body Mass Index
BP	Blood Pressure
bpm	Beats per Minute
BUN	Blood Urea Nitrogen
CBC	Complete Blood Count
CDC	Centers for Disease Control and Prevention
CFR	Code of Federal Regulations
C_{max}	Maximum Plasma Concentration
CMS	Clinical Materials Services
ConMed(s)	Concomitant Medication(s)
CPM	Clinical Project Manager
CROMS	Clinical Research Operations and Management Support
CSR	Clinical Study Report
CTU	Clinical Trial Unit
CV%	Coefficient of Variation
DCC	Data Coordinating Center
DMID	Division of Microbiology and Infectious Diseases
DNA	Deoxyribonucleic acid
eCRF	Electronic Case Report Form
EC	Ethics Committee
ECG	Electrocardiogram
EDC	Electronic Data Capture
ET	Early Termination

FDA	Food and Drug Administration
FSH	Follicle-stimulating hormone
FWA	Federalwide Assurance
g	Gram(s)
GCP	Good Clinical Practice
g/dL	Gram(s) per Deciliter
h	Hour(s)
HBsAg	Hepatitis B Surface Antigen
HCG (β-HCG)	Human Chorionic Gonadotropic Hormone
Hct	Hematocrit
Hgb	Hemoglobin
HCV	Hepatitis C Virus
HEENT	Head, Eyes, Ears, Nose, and Throat
HIV	Human Immunodeficiency Virus
HPLC-MS/MS	High-Performance Liquid Chromatography with Tandem Mass Spectrometry
HR	Heart Rate
IB	Investigator's Brochure
IC_{50}	50% Inhibitory Concentration
ICF	Informed Consent Form
ICH	International Conference on Harmonization
ICMJE	International Committee of Medical Journal Editors
IM	Intramuscular
IND	Investigational New Drug
IRB	Institutional Review Board
ISM	Independent Safety Monitor
IV	Intravenous
kg	Kilogram
LLN	Lower Limit of Normal
MDMA	3,4-methylenedioxy-methamphetamine
MedDRA®	Medical Dictionary for Regulatory Activities

MH	Medical History
µg	Microgram(s)
µM	Micromolar
µmol	Micromole(s)
mg	Milligram(s)
mg/dl	Milligram(s) per Deciliter
MIC	Minimum Inhibitory Concentration
mL	Milliliter(s)
mm	Millimeter(s)
MM	Medical monitor
MMA	Mouse Micronucleus Aberration Assay
mmHg	Millimeters of Mercury
min	Minute(s)
MOP	Manual of Procedures
msec	Milliseconds(s)
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
NOAEL	No observable adverse effect level
NSAIDs	Nonsteroidal Anti-Inflammatory Drugs
OCRR	Office of Clinical Research Resources
PD	Pharmacodynamic(s)
PE	Physical Examination
PI	Principal Investigator
PK	Pharmacokinetics
PO	Oral
POC	Point of Contact
PVG	Pharmacovigilance Group
QTc	Corrected QT Interval
RBC	Red Blood Cell
RP	Research Pharmacist
SAE	Serious Adverse Event

SAP	Statistical Analysis Plan
sec	Second(s)
SMC	Safety Monitoring Committee
T_{max}	Time to Maximum Plasma Concentration
TCA	Tricyclic antidepressants
UA	Urinalysis
VS	Vital Signs
ULN	Upper Limit of Normal
WBC	White Blood Cell Count
WHO	World Health Organization

PROTOCOL SUMMARY

Title:	A Phase 1 Clinical Trial to Evaluate the Plasma Pharmacokinetics (PK), Safety, and Tolerability of a Single Oral Dose of Zoliflodacin in Healthy Male and Female Subjects
Phase:	1
Population:	Eight healthy male and female subjects aged 18 to 45 years inclusive
Number of Sites:	One (IQVIA Phase One Services, LLC, Overland Park, KS)
Study Duration:	Approximately 4 weeks
Subject Participation Duration:	Up to 10 days (from dosing to final visit)
Description of Agents:	The study drug, zoliflodacin (also known as AZD0914 and ETX0914), is a spiropyrimidinetrione antibacterial agent. Its chemical name 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. It is formulated as a powder in sachets containing 2 grams (g) of the active agent and inactive excipients.
Objectives:	**Primary:** • To evaluate the plasma PK of zoliflodacin after administration of a single 4-g oral dose under fasting conditions **Secondary:** • To evaluate the safety and tolerability of a single 4-g oral dose of zoliflodacin
Outcome Measures:	**Primary:** C_{max}, T_{max}, $AUC_{(0\text{-last})}$, and other PK parameters for zoliflodacin determined using plasma concentrations of zoliflodacin in blood samples collected on Day 1 [baseline (30 min before dosing) and 0.5 h, 1 h, 2 h, 3 h, 4 h, 6 h, 8 h, and 12 h after dosing], Day 2 [24 h and 36 h after dosing], Day 3 [48 h after dosing], and Day 4 [72 h after dosing],

or early termination (ET), and measured using a validated HPLC-MS/MS method

Secondary:

- The occurrence of treatment-emergent serious adverse events (SAEs) from administration of zoliflodacin on Day 1 to Final Visit (Day 8 ± 2), or ET
- The occurrence of unsolicited treatment-emergent adverse events (AEs) from administration of zoliflodacin on Day 1 to Final Visit (Day 8 ± 2), or ET
- The changes from baseline (up to 60 min before dosing) in vital signs (VS) following administration of zoliflodacin, as measured on Day 1 [1 h, 2 h, and 4 h after dosing], Days 2, 3, and 4 [24 h, 48 h, and 72 h, respectively, after dosing], and Final Visit (Day 8 ± 2), or ET
- The changes from baseline (Day -1) in clinical laboratory values following administration of zoliflodacin, as measured on Day 4, or ET
- The changes from baseline (up to 60 min before dosing) in electrocardiogram (ECG) parameters following administration of zoliflodacin, as measured on Day 1 [1 h, 2 h, and 4 h after dosing] and Day 4 [72 h after dosing], or ET

Description of Study Design:

The trial is an open-label, non-randomized evaluation of the PK and safety profiles of a single 4-g oral dose of zoliflodacin in eight healthy male or female subjects. Each subject will receive zoliflodacin reconstituted in [redacted] after at least an 8-h fast, which will continue for at least 4 h after dosing. Consumption of water is permitted during the fasting period. All subjects will be dosed in the morning of Day 1 in a staggered fashion with a minimum of several minutes apart.

Subjects who consent to participate will be enrolled in the trial if they meet all of the inclusion and none of the exclusion eligibility criteria. Laboratory assessments at Screening Visit will include testing for serology [human immunodeficiency virus (HIV), hepatitis B surface antigen (HBsAg), and hepatitis C virus (HCV)], drugs of abuse, and alcohol. Serum pregnancy testing will be done in all females and serum FSH levels will be measured only in post-menopausal females. Safety assessments at scheduled visits will include medical history (MH), vital signs (VS), physical examination (PE), 12-lead ECG, and clinical laboratory values including complete blood count (CBC) with differential, comprehensive metabolic (chemistry) panel, and urinalysis (UA) for blood, glucose, and protein. Treatment-emergent AEs and SAEs will be assessed from the time of dosing to the end of the trial. Plasma

	for PK analysis will be collected at scheduled time points before and after dosing on Day 1 and through Day 4, or ET.
Estimated Time to Complete Enrollment:	Up to 10 days (from dosing to last visit)
Halting Criteria Decision Process:	If criteria for halting the trial (as listed in Section 9.5.1) are met during dosing on Day 1 prior to all subjects being dosed, an *ad hoc* Safety Monitoring Committee (SMC) meeting will be held to review all available safety data and to make recommendations about the dosing of all further subjects in the trial. A scheduled SMC meeting will be held after all subjects complete the trial to review all safety data, and advise on using the 4-g dose in a subsequent thorough QTc study.

1 KEY ROLES

Program Principal Investigator: **George A Saviolakis, MD, PhD**
Medical Director
DynPort Vaccine Company, LLC
110 Thomas Johnson Drive, Suite 200
Frederick, MD 21702
Office: 240-651-8116
Mobile: [redacted]
Email: George.Saviolakis@csra.com

Site Principal Investigator: **Barbara Lomeli, MD**
IQVIA™
6700 West 115th Street
Overland Park, KS 66211
Office: 913-708-6999
Mobile: [redacted]
Email: barbara.lomeli@Quintilesims.com

DMID Clinical Project Manager: **Meg Gordon, RN**
OCRR/DMID/NIAID/NIH
5601 Fishers Lane, Room 7G37
Bethesda, MD 20892-9826
Office: 301-761-7604
Mobile: [redacted]
Email: meg.gordon@nih.gov

DMID Medical Officer: **Rick Fairhurst, MD, PhD**
OCRR/DMID/NIAID/NIH
5601 Fishers Lane, Room 8B22
Bethesda, MD 20892-9826
Office: 301-761-5077
Mobile: [redacted]
Email: rfairhurst@niaid.nih.gov

DMID Medical Monitor:	**Soju Chang, MD** OCRR/DMID/NIAID/NIH 5601 Fishers Lane, Room 7E59 Bethesda, MD 20892-9826 Office: 240-292-4178 Email: changsoju@niaid.nih.gov
Pharmaceutical Sponsor Medical Monitor:	**Robin Isaacs, MD** Chief Medical Officer Entasis Therapeutics [redacted]
Safety and Pharmacovigilance Contractor:	**Pharmacovigilance Group** CROMS/DMID/NIAID/NIH 6500 Rock Spring Drive, Suite 650 Bethesda, MD 20817 SAE Hot Line: 1-800-537-9979 SAE Fax: 1-800-275-7619 SAE Email: PVG@dmidcroms.com
Data Coordinating Center:	**The Emmes Corporation** 401 N. Washington Street, Suite 700 Rockville, MD 20850 Phone: 301-251-1161 Fax: 301-576-3558 Email: phase1@emmes.com

Study Agent Repository:	**Fisher BioServices** c/o DMID Clinical Materials Services (CMS) 20439 Seneca Meadows Parkway Germantown, MD 20876 Phone: 240-477-1350 Fax: 240-477-1360 Email: DMID.CMS@thermofisher.com
Clinical Laboratory:	**IQVIA Early Clinical Development Laboratory** 6700 West 115th Street Overland Park, KS 66211 Phone: 913-708-6023 Fax: 913-800-6564
Bioanalytical Laboratory:	**KCAS Bioanalytical Services** 12400 Shawnee Mission Parkway Shawnee, KS 66216 POC: Marsha Luna Senior Manager, PBI Phone: 913-248-3042 Email: marsha.luna@kcasbio.com

2 BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 Background Information

2.1.1 Overview of the Disease: Gonorrhea and its Treatment

Neisseria gonorrhoeae, currently the second most common bacterial sexually transmitted infection worldwide (after *Chlamydia trachomatis*), is a serious public health problem.[1,2] In 2008, the World Health Organization (WHO) estimated 106 million cases of gonorrhea among adults worldwide.[3] In 2012, the Centers for Disease Control and Prevention (CDC) reported 311,404 cases of gonorrhea in the US; however, due to incomplete reporting, up to 820,000 cases may occur annually.[4] *N. gonorrhoeae* has developed resistance to all antimicrobial treatments for gonorrhea. The CDC recently released its report "Antibiotic Resistance Threats in the US, 2013," which ranks drug-resistant *N. gonorrhoeae* as an "Urgent Threat," defined as "an immediate public health threat that requires urgent and aggressive action."[5] This level of resistance has demanded alternative oral treatments, which until recently have been readily available.[6] Examples of drug classes that are widely used to treat gonorrhea, but are no longer recommended as monotherapy due to resistance, include sulfanilamides, penicillin, tetracyclines, and fluoroquinolones. [1,2] Most recently, *N. gonorrhoeae* resistance to macrolides (including azithromycin), cefixime, and ceftriaxone, and consequent clinical failures, have been reported. Since two extended-spectrum cephalosporins, ceftriaxone and cefixime, have recently been the only first-line options for treating gonorrhea, resistance and treatment failures with these drugs are particularly concerning. It is only a matter of time before gonococci with full resistance to extended-spectrum cephalosporins emerge and spread globally.[7] The threat of widespread ceftriaxone resistance and untreatable *N. gonorrhoeae* infection is real.[5,6] The clinical development of zoliflodacin (also known as AZD0914 and ETX0914) addresses the need for new antibiotics that can be used alone or with other agents to treat uncomplicated gonorrhea caused by emergent *N. gonorrhoeae* strains that are resistant to existing antibiotics.[8,9]

2.1.2 Zoliflodacin Development

Zoliflodacin is a spiropyrimidinetrione antibacterial drug, which inhibits bacterial DNA synthesis by a novel mechanism. Zoliflodacin demonstrates *in vitro* activity against *N. gonorrhoeae* strains that are susceptible or resistant to current available therapies and *in vivo* efficacy, including in patients with uncomplicated gonorrhea. [8,9,10,11]

This section provides a summary of zoliflodacin. Nonclinical and clinical data are provided in more detail in the Investigator's Brochure (IB).

Chemistry

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.

Mechanism of Action

Zoliflodacin inhibits bacterial Type II topoisomerases, specifically bacterial gyrases. The activity and mode of inhibition of bacterial gyrase by zoliflodacin has been assessed in several *in vitro* assays. Zoliflodacin stabilizes the [redacted], but in addition prevents its [redacted] is further supported [redacted] *N. gonorrhoeae* resistance to [redacted].[8]

Microbiological Activity

In vitro: Zoliflodacin has demonstrated activity *in vitro* against *N. gonorrhoeae*, including strains that are resistant to penicillin, ciprofloxacin, tetracycline, azithromycin, ceftriaxone, and cefixime. Minimum inhibitory concentration $(MIC)_{90}$ values ranged from 0.125 to 0.25 µg/mL. Zoliflodacin has also shown bactericidal activity at an MIC_{90} of 0.25 µg/mL against *C. trachomatis*, which often co-infects patients with gonorrhea.[8,9,10]

In vivo: Mouse model of Staphylococcus aureus infection: An animal model for *N. gonorrhoeae* has not been established due to spontaneous bacterial eradication. To estimate a human efficacious exposure of zoliflodacin for *N. gonorrhoeae*, a surrogate pathogen approach, which utilizes pharmacokinetics/pharmacodynamics (PK/PD) determinations from a *S. aureus* neutropenic mouse thigh model that correlates with human clinical efficacy, was used. In this model, zoliflodacin was efficacious *in vivo* against clinical *S. aureus* isolates. The MIC values of zoliflodacin against *S. aureus* and *N. gonorrhoeae* and the clinical doses suggest that efficacy can be translated across pathogens based on AUC/MIC targets. From this analysis, the efficacious human exposure for zoliflodacin was estimated utilizing a PK/PD target that covers a mean fAUC/MIC of 66 (range 43–98) for *S. aureus* in the mouse thigh model. This target, combined with the MIC_{90} of zoliflodacin for *N. gonorrhoeae* and the human unbound fraction (f_u) of 17%, translated to a predicted efficacious mean AUC in humans of 49 µg*h/mL.[8,9]

In vivo: Human Phase 2 trial: Zoliflodacin was recently investigated in a multi-center, randomized, Phase 2 trial to assess the safety and efficacy of zoliflodacin administered orally to adults to treat uncomplicated urogenital gonorrhea compared to treatment with ceftriaxone administered intramuscularly. Subjects were randomly assigned to receive a single oral dose of 2 g of zoliflodacin, 3 g of zoliflodacin, or a single intramuscular (IM) dose of 500 mg of

ceftriaxone. In the per-protocol population, microbiological cure was observed [redacted].[8]

Human Pharmacokinetics

A single-center, multi-part, Phase 1 trial with an oral suspension of zoliflodacin versus placebo was conducted in healthy men and women. In part A, 48 subjects received single ascending doses of zoliflodacin ranging from 200 mg to 4 g, or placebo (6:2, zoliflodacin:placebo). In Part B, zoliflodacin was administered in doses of 1.5 g (N=8) and 3 g (N=10) to evaluate the effect of food on the PK of zoliflodacin. Zoliflodacin was absorbed relatively quickly under fasting conditions, with a median T_{max} ranging from [redacted]. Following C_{max}, plasma drug concentrations [redacted]. The terminal elimination phase started at approximately [redacted], zoliflodacin concentrations declined with a half-life ranging from [redacted]. Exposures [redacted], suggesting a [redacted]. When [redacted] of zoliflodacin was administered, a [redacted] was seen if the drug was [redacted] The C_{max} [redacted] consistent with [redacted].[8]

In a Phase 2 trial of patients with gonorrhea, [redacted]. The AUC and C_{max} values for zoliflodacin at the various doses also point to a margin of safety, as seen by multiples to rat and dog NOAEL (no observable adverse effect level) values.[8]

Potential for Drug-Drug Interactions

Zoliflodacin is a [redacted] in preclinical studies. *In vitro* and *in vivo* metabolism across species indicate that the compound is [redacted]. As a single dose therapy with no evidence of [redacted], the compound is not expected to show lasting effects. The potential for drug-drug interactions has been evaluated using a physiologically-based PK model developed with the [redacted].[8] In summary:

1. Zoliflodacin as a victim: Single doses of zoliflodacin ranging from [redacted]. The primary

PK driver for efficacy of zoliflodacin is [redacted].

2. Zoliflodacin as a perpetrator: Zoliflodacin is predicted to [redacted]. The extent of the interaction is not predicted to differ between the zoliflodacin [redacted]. This [redacted] administration of zoliflodacin.

Safety

In vitro studies: There was no [redacted] There was no inhibition of [redacted]

Some fluoroquinolones induce QT prolongation leading to arrhythmias and torsades de pointes, which have been correlated with binding to the hERG K+ channel. In contrast, zoliflodacin showed [redacted]

Animal pharmacology and toxicology studies: Zoliflodacin was well tolerated – as a [redacted]. There was [redacted] At this highest dose tested, [redacted] These effects were [redacted] There were [redacted]

[redacted].[8]

Effects of zoliflodacin on female fertility and embryofetal development have been assessed [redacted] Effects on [redacted] Following administration of zoliflodacin at doses of [redacted]. At all dose levels, there were [redacted]

[redacted]

The effects of zoliflodacin on male fertility have been assessed [redacted] Administration at [redacted].

Human trials: Three trials have evaluated the safety of zoliflodacin in humans.[8]

In healthy subjects in the Phase 1 trials, [redacted].[8,12]

In patients in the Phase 2 trial, zoliflodacin was generally safe and well tolerated. AEs reported were generally mild, were non-serious, and none led to trial discontinuation. The AE and laboratory safety profiles of zoliflodacin were generally similar to those of ceftriaxone. The AE profile of zoliflodacin 2 g was generally similar to that of zoliflodacin 3 g; headache was more common in the zoliflodacin 3-g group (9%) compared to the zoliflodacin 2-g group (0%). Dysgeusia was not reported in the Phase 2 trial. Vomiting was reported by one patient receiving zoliflodacin 3 g.[8,13]

There were no AEs reported at a frequency of ≥5% when the AE profiles of the zoliflodacin 2-g and zoliflodacin 3-g groups were combined. The most common AEs reported with zoliflodacin (≥3% based on the zoliflodacin 2-g and zoliflodacin 3g groups combined and excluding infection-related AEs) were diarrhea, headache, and nausea. [8,13]

2.2 Rationale

2.2.1 Rationale for Use of Zoliflodacin

A single PO dose of zoliflodacin has been shown to be effective against uncomplicated gonorrhea caused by *N. gonorrhoeae* strains susceptible or resistant to currently available therapies. These findings supported the ongoing clinical development of zoliflodacin as a potential new therapeutic option for uncomplicated gonorrhea. The recently completed Phase 2 clinical trial suggests that zoliflodacin doses in the range of 2 g to 3 g are therapeutic. The present clinical trial will further characterize the PK characteristics and safety profile of a high 4-g dose of zoliflodacin and evaluate whether it can be used in a planned thorough QTc study.

2.2.2 Rationale for Dose Selection

Regulatory guidance ICH/FDA E14 has emphasized the need to obtain robust data on the effect of new chemical entities on ECG parameters, focusing on cardiac repolarization as measured by the QTc.[14] To obtain these data, the potential of a single therapeutic dose and a single supratherapeutic dose of the study drug to prolong cardiac repolarization is measured. The 4-g dose of a previous formulation of zoliflodacin was found to be safe and well tolerated in a Phase 1 trial. The current trial will further characterize the PK and safety profiles of the 4-g dose of a new zoliflodacin formulation. This dose could result in drug exposures that are multiples of the proposed 2-g therapeutic dose and meet the requirements for a supratherapeutic dose according to the ICH/FDA E14 Guidance for thorough QTc studies. [14]

2.3 Identified and Potential Risks and Benefits

2.3.1 Identified Risks

Single zoliflodacin doses ranging from 200 mg to 4 g were generally safe and well tolerated in the Phase 1 and Phase 2 clinical trials conducted to date in healthy subjects and patients with uncomplicated gonorrhea.[8,12,13] The most common AEs reported with zoliflodacin in the Phase 1 trials were dysgeusia and headache, and in the Phase 2 trial (≥3% based on the zoliflodacin 2-g and zoliflodacin 3-g groups combined and excluding infection-related AEs) were diarrhea, headache, and nausea. These events will continue to be monitored and evaluated.

2.3.2 Potential Risks

The potential risks to subjects participating in the trial are those related to PO dosing of zoliflodacin, and associated study procedures (venipuncture, IV catheter insertion, ECG patch application, and blood sampling).

Zoliflodacin-Associated Risks

As for any drug, exposure to zoliflodacin could result in an allergic or hypersensitivity reaction that could be mild or life-threatening. Should a reaction occur, medically-appropriate diagnostic and therapeutic measures will be taken immediately by trained site clinicians and staff.

In a Phase 1 trial, analysis of [redacted]. Monitoring of [redacted] will continue in the trial.

In a Phase 1 and Phase 2 trial, no trends [redacted]. Standard clinical monitoring of [redacted] The incidence of these findings typically [redacted] in the first-in-human trial. [redacted]. These findings are not considered to preclude administration of zoliflodacin to healthy male subjects with appropriate informed consent.

Effects of chronic dosing of zoliflodacin on [redacted]. With respect to [redacted]. The effects of zoliflodacin [redacted] Based on these preclinical data and the gonorrheal clinical indication, in current and future clinical trials:

- Female subjects will be pregnancy tested prior to administration of zoliflodacin and at the end of the trial and will be required to use specified birth control methods for 30 days following final study visit.

- Male subjects will be required to use specified birth control methods for 90 days following final study visit and will be advised not to donate sperm during this period.

Venipuncture and IV Catheter Placement Risks

Venipuncture: Venipuncture causes transient discomfort and may cause fainting. Bruising at the site of venipuncture may occur, but can be prevented or lessened by applying pressure for several min. Infection at the site is possible but highly unlikely as aseptic technique will be used.

IV catheter placement: An indwelling catheter may be placed in an arm vein (preferably antecubital) for frequent blood drawing for PK measurements. The catheter may cause phlebitis with signs of redness and warmth at or near the IV insertion site, and thrombophlebitis with a hard area palpable near the IV insertion site. These risks are minimal as the IV catheters, when used, are only used briefly after dosing. Careful inspection of the catheter site, including visualization of blood return, and withdrawal of the catheter if needed will minimize this risk. There is a risk of infection; however, this is a small risk as aseptic technique will be used.

Additional Risks

ECG: Possible side effects from ECG patches include a rash or minor irritation of the skin.

Blood draws: The amount of blood drawn is about 18 mL during the Screening Visit and Day -1 period, 74 mL during the inpatient period (Days 1-4), and 92 mL during the entire trial (Appendix D). Additionally, small amounts of blood loss may occur if an IV catheter is used or additional blood samples are collected (for repeat laboratory testing, evaluation of AE, etc.). Overall, the amount of blood that may be drawn during the trial is within the amount that considered safe to be drawn during short or extended periods, respectively, and not excessive for the safety and PK assessment requirements of Phase 1 trials. However, there is a small risk that some subjects may develop mild symptoms of hypovolemia or anemia during the trial. These are reversible with specific treatment (fluid replacement, good nutrition, vitamins, or iron supplementation).

2.3.3 Known Potential Benefits

The trial has no benefit for the subjects participating in the trial. Knowledge gained in the trial could be of benefit to public health and to individuals with gonorrhea or at risk of acquiring it.

3 OBJECTIVES AND OUTCOME MEASURES

3.1 Study Objectives

3.1.1 Primary

- To evaluate the plasma PK of zoliflodacin after administration of a single 4-g oral dose under fasting conditions

3.1.2 Secondary

- To evaluate the safety and tolerability of a single 4-g oral dose of zoliflodacin

3.2 Study Outcome Measures

3.2.1 Primary

- C_{max}, T_{max}, $AUC_{(0\text{-last})}$, and other PK parameters for zoliflodacin determined using plasma concentrations of zoliflodacin in blood samples collected on Day 1 [baseline (30 min before dosing) and 0.5 h, 1 h, 2 h, 3 h, 4 h, 6 h, 8 h, and 12 h after dosing], Day 2 [24 h and 36 h after dosing], Day 3 [48 h after dosing], and Day 4 [72 h after dosing], or ET, and measured using a validated HPLC-MS/MS method

3.2.2 Secondary

- The occurrence of treatment-emergent SAEs from administration of zoliflodacin on Day 1 to Final Visit (Day 8 ± 2), or ET
- The occurrence of unsolicited treatment-emergent AEs from administration of zoliflodacin on Day 1 to Final Visit (Day 8 ± 2), or ET
- The changes from baseline (up to 60 min before dosing) in VS following administration of zoliflodacin, as measured on Day 1 [1 h, 2 h, and 4 h after dosing], Days 2, 3, and 4 [24 h, 48 h, and 72 h, respectively, after dosing], and Final Visit (Day 8 ± 2), or ET
- The changes from baseline (Day -1) in clinical laboratory values following administration of zoliflodacin, as measured on Day 4, or ET
- The changes from baseline (up to 60 min before dosing) in ECG parameters following administration of zoliflodacin, as measured on Day 1 [1 h, 2 h, and 4 h after dosing] and Day 4 [72 h after dosing], or ET

4 STUDY DESIGN

The trial will be performed as an open-label, non-randomized, single-dose design in eight healthy male or female subjects to evaluate the PK and safety profiles of the zoliflodacin formulation. All subjects will be dosed in the morning of Day 1 in a staggered fashion with a minimum of several minutes apart. Each subject will receive a single 4g dose of zoliflodacin (2 x 2 g sachets of zoliflodacin) after at least an 8-h fast, which will continue for at least 4 h after dosing. Consumption of water will be permitted during the fasting period. Subjects will be monitored as inpatients in the Clinical Trial Unit (CTU) up to Day 4 and at the Final Visit (Day 8 ± 2).

Subjects who consent to participate will be enrolled in the trial if they meet all inclusion and none of the exclusion eligibility criteria. Laboratory assessments at Screening Visit will include testing for serology (HIV, HBsAg, and HCV), drugs of abuse, and alcohol. Serum pregnancy testing will be done in all women and serum FSH levels will be measured only in post-menopausal women. Safety assessments at scheduled visits will include MH, VS, PE, 12-lead ECG, and clinical laboratory tests including CBC with differential, comprehensive metabolic (chemistry) panel, and UA. Treatment-emergent AEs and SAEs will be assessed from the time of dosing to the end of the trial. Plasma for PK analysis will be collected at scheduled time points before and after initiation of dosing on Day 1 and through Day 4, or ET.

Safety Monitoring and SMC Role

A SMC will be appointed to oversee the safe conduct of the trial. A scheduled SMC meeting will be held after all subjects complete the trial to review safety data, and advise on using the 4-g dose in a subsequent thorough QTc study. If criteria for halting the trial (as listed in Section 9.5.1) are met during dosing on Day 1, an *ad hoc* SMC meeting will be held to review all available safety data and to make recommendations about the dosing of all further subjects in the trial. An independent safety monitor (ISM), who is local to the CTU, will review SAEs and other severe safety signals and provide an independent analysis to the Site PI, SMC, and DMID.

4.1 Sub-studies

No sub-studies are planned.

5 STUDY ENROLLMENT AND WITHDRAWAL

Only subjects who meet all inclusion criteria and none of the exclusion criteria will be eligible for enrollment into the trial. No exemptions are granted on Inclusion/Exclusion Criteria in DMID sponsored trials.

Eight healthy male and female subjects, aged 18 to 45 years inclusive, will be enrolled in a single cohort. Up to two alternates will be recruited.

5.1 Subject Inclusion Criteria

All must be answered YES for the subject to be eligible for study participation:

1) Informed consent form (ICF) understood and signed before initiating any study procedures
2) Healthy male or female, as assessed by the authorized site clinician (listed on FDA Form 1572)
3) Willingness to comply with and be available for all protocol procedures including inpatient confinement for about 4 days and availability for follow-up for the duration of the trial
4) Aged 18 to 45 years inclusive on the day of study drug dosing
5) Body Mass Index (BMI) ≥18.5 and ≤30 kg/m^2 and weight ≥50 kg (110 lbs.) and ≤100 kg (220 lbs.)
6) In female subjects of childbearing potential, a negative serum pregnancy test at Screening Visit and on Day -1
    - *Note: A woman is considered of childbearing potential unless post-menopausal (≥ 1 year without menses without other known or suspected cause and with a FSH level in the menopausal range), or surgically sterilized (hysterectomy, salpingectomy, oophorectomy or tubal ligation/occlusion)*
7) If female, not pregnant, not breast feeding, and not planning on becoming pregnant during the trial and for 30 days after study participation
8) Females of childbearing potential and males agree to use acceptable contraception for the duration of the trial and for 30 days (females) or 90 days (males) after final study visit
    - *Note: A highly effective method of birth control is defined as one with a low failure rate (i.e., less than 1 percent per year) according to the CDC criteria.[15] These include progestin implants, intrauterine devices (IUDs), surgical (hysterectomy, salpingectomy, oophorectomy or tubal ligation/occlusion; vasectomy), or abstinence.*

Use of methods with higher failure rate (such as progestin injectables, combined oral hormonal contraceptives, condoms, and diaphragms) will not be acceptable when used alone, but they could be considered if used in combination with another method (e.g., a female using combined oral contraceptives if her male partner is sterile, or if she and her non-sterile male partner use a double-barrier method), after consultation with the DMID Medical Officer.

9) Male subjects must agree to refrain from sperm donation for the duration of the trial and for 90 days after Final Visit

10) Laboratory tests, as outlined in Section 8.2.1.1, are in the normal reference range with acceptable exceptions as noted in Section 8.2.1.1 and Appendix B

11) VS, as outlined in Section 8.1.6, are within the acceptable range per Appendix B

12) Has adequate venous access for blood collection

13) Urine drug screen is negative for tested substances (see Section 8.2.1.5)

14) Alcohol test (breathalyzer) is negative

15) Willing to abstain from alcohol consumption for 2 days before Day -1 and during the trial

5.2 Subject Exclusion Criteria

All must be answered NO for the subject to be eligible for study participation:

1) History of a chronic medical or surgical condition that would interfere with the accurate assessment of the trial's objectives or increase the subject's risk profile

   - *Note: Chronic medical conditions include: diabetes mellitus; asthma requiring use of medication in the year before screening; autoimmune disorder such as lupus erythematosus, Wegener's, rheumatoid arthritis, thyroid disease; cardiovascular disease, including coronary artery disease or cerebrovascular disease, or surgery; syncope related to cardiac arrhythmia or unexplained; chronic hypertension; malignancy except low-grade (squamous and basal cell) skin cancer thought to be cured; chronic renal, hepatic, pulmonary, or endocrine disease, myopathy, or neuropathy; gastrointestinal or biliary surgery.*

2) History of hypersensitivity or severe allergic reaction of any type to medications, bee stings, food, or environmental factors

   - *Note: Severe allergic reaction is defined as any of the following: anaphylaxis, urticaria, or angioedema*

3) Active allergic symptoms to seasonal and animal allergens that require treatment

4) A marked baseline prolongation of ECG intervals, or HR <45 bpm or >100 bpm on ECG measurements
   - *Note: The following are considered prolonged ECG intervals: QTc/QTcF >449 msec in males and females; PR >209 msec; and QRS >110 msec*

5) Clinically significant abnormal ECG results

   Note: Clinically significant abnormal ECG results include: complete left or right bundle branch block; other ventricular conduction block; 2nd degree or 3rd degree atrioventricular (AV) block; sustained atrial or ventricular arrhythmia; two premature ventricular contractions in a row; pattern of ST elevation felt consistent with cardiac ischemia; evidence of a previous myocardial infarction (MI), left ventricular hypertrophy (LVH), or more than minor non-specific ST-T wave changes; or any condition deemed clinically significant by a study investigator.

6) Abnormal renal function
   Note: Normal renal function is defined as normal creatinine [per criteria in Appendix B] and normal estimated glomerular filtration rate (eGFR) [i.e., >80.0 mL/min] values according to Cockroft-Gault.

7) Positive serology results for HIV, HBsAg, or HCV

8) Febrile illness with temperature >37.6°C for <7 days before dosing

9) Donated whole blood or blood products within 60 days before dosing, or plans to donate before Final Visit (Day 8 ± 2)

   • *Note: Blood products include RBCs, WBCs, platelets, and plasma*

10) Known allergic reactions to any of the study drug components present in the formulation or in its processing, as listed in the IB

11) Treatment with another investigational product within 30 days of dosing or 5 half-lives or twice the duration of the biological effect of the study drug (whichever is longer)

   • *Note: Investigational products include a drug, vaccine, biologic, device or blood product*

12) Active drug or alcohol use, abuse, or dependence within 12 months before Screening Visit that, in the opinion of the investigator, would interfere with adherence to study requirements

13) Use of any prescription medication within 30 days before dosing or planned use during the study period except as noted below and approved by the designated study clinician

   - *Note 1: Prohibited medications include moderate or strong CYP3A4 inducers (per Section 6.9); antibiotics; injectable or oral antidiabetic drugs; anti-lipid drugs;*

immunosuppressive agents; immune modulators; oral corticosteroids; anti-neoplastic agents; any vaccine (licensed or investigational) except licensed influenza vaccine during the flu season, which is allowed 7 days before or after dosing.

- *Note 2: Allowed medications include: oral contraceptives; H1 antihistamines; topical/ intranasal corticosteroids; nonsteroidal anti-inflammatory drugs [NSAIDS]; licensed influenza vaccine during the flu season, 7 days before or after dosing.*

14) Use of any non-prescription medication, herbal preparation, or nutritional supplement within 15 days before dosing or planned use during the study unless approved by the study clinician

    - *Note: Exceptions: St. John's wart is not allowed within 30 days of dosing, vitamins and OTC medications taken for a brief period (<48 h) for the treatment of common symptoms (such as headache, indigestion, muscle pain) may be allowed as approved by the designated study clinician.*

15) Intake of caffeinated beverages or food within 72 h before dosing or a history of high caffeine consumption (e.g., in the last 4 months drinking >5 cups of coffee/day)

16) Smoking or use of tobacco or nicotine-containing products within 15 days before dosing

17) Engagement in strenuous exercise within 15 days before dosing (e.g., marathon running, long distance cycling, weight lifting) and during the study period

18) Any specific behavioral or clinical condition that in the judgment of the investigator precludes participation because it could affect compliance with study procedures or subject safety

19) Plans to enroll or is already enrolled in another clinical trial that could interfere with safety assessment of the study drug at any time during the study period

    - *Note: Includes trials that have a study intervention such as a drug, biologic, or device.*

20) Is a study site employee or staff member who is paid entirely or partially by the OCRR/NIAID contract for the DMID-funded trial

    - *Note: Site employees or staff include the PIs, sub-investigators, or staff who are supervised by the PI or sub-investigators.*

5.3 Treatment Assignment Procedures

5.3.1 Enrollment Procedures

This is a Phase 1, open-label clinical trial of a single dose of zoliflodacin. Eight healthy subjects who consent to participate in the trial and meet the eligibility criteria will be enrolled following admittance to the CTU and confirmation of eligibility. Subjects will be registered using a web-based application developed by The Emmes Corporation, the Data Coordinating Center (DCC) for the trial.

Per ICH guideline E6: GCP, screening records will be kept at the participating site to document the reason why an individual was screened but did not meet trial entry criteria, by recording it in the DCC AdvantageEDC^{SM} (Electronic Data Capture System).

Subjects will be enrolled online using the enrollment module of AdvantageEDC^{SM} after the demographic and eligibility data have been entered into the system.

Instructions for using the enrollment module are included in the AdvantageEDC^{SM} User's Guide.

5.3.2 Masking Procedures

This is an open-label clinical trial. The study staff participating in the administration of the study drug and assessment of subjects will be aware of the administered study drug.

The SMC will review aggregate data in the open session. Grouped data will be reviewed by the SMC in the closed session only.

5.3.3 Reasons for Withdrawal and Discontinuation of Study Product Administration

A subject may withdraw from the trial at any time for any reason, without any consequences.

A study subject will be discontinued from participation in the trial if any of the following reasons occur before dosing:

- Request by the subject to terminate participation;
- Failure to receive the study drug due to difficulty ingesting it.

A subject may be removed from the trial after dosing for the following reasons; however, whenever possible the subject should be followed for safety per protocol:

- Failure to adhere to protocol requirements;
- Loss to follow-up;
- Request of primary care provider;
- Request of the Institutional Review Board (IRB)/Ethics Committee (EC), Food and Drug Administration (FDA), or DMID;

- The subject's well-being, based on the opinion of the investigator;
- The occurrence of an SAE or AE warranting withdrawal;
- Failure to ingest the entire volume of drug suspension;
- Vomiting within 24 h after dosing.

5.3.4 Handling of Withdrawals and Discontinuation of Administration

Subjects who are withdrawn before dosing may be replaced. Following dosing, one subject may withdraw before Day 4 without being replaced. A subject who cannot ingest the entire volume of drug suspension or a subject who took the entire dose but vomits up to 24 h after dosing will also be replaced. Subjects who ingest the entire volume of the initial [redacted] drug suspension but who do not ingest the content of the second [redacted] study drug suspension will not be withdrawn from the study or replaced. Subjects who received any amount of the study drug but withdraw from the trial will be encouraged to continue follow-up (with subjects' consent) for safety assessments and PK sample collection. Subjects withdrawing will be asked to complete a final termination visit if they do not wish to be followed per protocol.

5.3.5 Lost to Follow-up

In the case of subjects who fail to appear for a follow-up assessment, extensive effort (i.e., three documented contact attempts via phone calls, e-mail, etc., made on separate occasions and followed by a certified letter) will be made to locate or recall them, or at least to determine their health status. These efforts will be documented in the subjects' records.

5.3.6 Termination of Study

Although the sponsor (DMID) has every intention of completing the trial, it reserves the right to terminate the trial at any time for clinical or administrative reasons. In addition, the trial may be terminated or suspended at the request of the FDA, SMC, or IRB/EC.

6 STUDY INTERVENTION/INVESTIGATIONAL PRODUCT

6.1 Study Product Description

The chemical name for zoliflodacin (also known as ETX0914 and AZD0914) 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 a spiropyrimidinetrione antibacterial agent, with a novel mode of action. It is a first-in-class oral gyrase inhibitor being developed for treatment of uncomplicated gonococcal infection, including cases caused by isolates with resistance to currently available treatments.

6.1.1 Acquisition

Zoliflodacin sachets are currently stored at a GMP facility. Upon request by DMID, the study drug will be shipped to the following address:

DMID-Clinical Materials Services (CMS)
Fisher BioServices
20439 Seneca Meadows Parkway
Germantown, MD 20876
Tel: 240-477-1350
Fax: 240-477-1360
E-mail: DMID.CMS@ThermoFisher.com

The study drug will be shipped from DMID-CMS to the CTU upon request and approval by DMID. Details will be provided in the protocol-specific Manual of Procedures (MOP).

6.1.2 Formulation, Packaging, and Labeling

Zoliflodacin is presented as granules for oral suspension, 50 % wt/wt, packaged in a single sachet. Each sachet contains 2 g spray-dried Zoliflodacin, co-formulated with [redacted].

Each sachet will be labeled in compliance with applicable regulatory requirements, including the FDA-required cautionary statement *"Caution- New drug -Limited by Federal (or United States) Law to Investigational Use Only."*

6.1.3 Product Storage and Stability

The zoliflodacin granules for oral suspension, 50% (w/w) should be stored at 2-8°C in the primary packaging.

The clinical supplies storage area at the site must be monitored by the site staff for temperature consistency with the acceptable storage temperature range specified in this protocol or in the study drug label. Documentation of temperature monitoring should be maintained. Further details are described in the MOP.

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

All subjects will receive a single 4-g PO dose (2 sachets of 2 g) of zoliflodacin in the morning of Day 1 in a staggered fashion several minutes apart.

The study drug will be inspected for damage, contamination, discoloration, or particulate matter before use. Any study drug that fails inspection should be quarantined at 2-8°C (35.6-46.4°F) and labeled 'Do Not Use' until further notice. The Site PI or responsible person should immediately contact the DMID Product Support Team at DMIDProductSupportTeam@niaid.nih.gov and DMID Clinical Project Manager (CPM) for further instructions before administering any additional study drug. Based on the information collected, DMID and/or Entasis will determine whether the affected study drug can be used. If it cannot be used, the site will receive specific instructions on how to return it to DMID CMS or destroy it on site.

A detailed procedure will be described in the MOP.

Eligible subjects who provide informed consent, undergo screening procedures, and qualify, meeting all inclusion and none of the exclusion criteria for the trial, will receive a single 4-g dose (2 sachets of 2 g of zoliflodacin reconstituted on the same day and administered in a total of [redacted]) orally after an overnight fast. The dose will be administered in a standardized cup as described in the MOP. After the [redacted] of zoliflodacin is administered, an additional [redacted] will be added to the same cup and consumed by the subject to chase the initial dose. Dosing should be completed within 5 minutes after each study product suspension. After dosing, subjects will continue to fast for an additional 4 h while water is allowed *ad lib*.

Refer to the protocol-specific MOP for detailed information on the preparation and administration of zoliflodacin.

6.3 Modification of Study Intervention/Investigational Product for a Subject

Not applicable for a single cohort study. See Study Halting Criteria, Section 9.5.1

6.3.1 Overdose

An overdose is defined as a dose greater than the high-dose level evaluated in the trial. All overdoses should be reported; if the overdose is associated with an AE, then the AE should also be reported. In the event of an overdose of zoliflodacin, the investigator should use clinical judgment in treating the overdose and contact the DMID Medical Monitor (MM). The investigator should refer to the relevant document(s) for detailed information regarding warnings, precautions, contraindications, AEs, and other significant data pertaining to zoliflodacin. Such documentation may include but not be limited to the IB.

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

The Site PI is responsible for the distribution and disposition of the study drug, and has ultimate responsibility for accountability. The Site PI may delegate this responsibility to the Site RP. If delegated, the Site RP will be responsible for maintaining complete records and documentation of the study drug's receipt, accountability, dispensation, temperature monitoring, storage conditions, and final disposition.

All study drugs, whether administered or not, must be documented on the appropriate study drug accountability record or dispensing log. Used and unused zoliflodacin sachets will be retained until monitored and released for disposition per DMID requirements.

Upon completion of the trial and after the final monitoring visit, any remaining unused study drug will either be returned or destroyed appropriately at the clinical site as per sponsor requirements and instructions, or in accordance with disposition plans.

6.5 Assessment of Subject Compliance with Study Intervention/ Investigational Product/Device

Since each dose of zoliflodacin will be administered by site personnel, subject compliance is not anticipated to be an issue. Complete information regarding any partial or interrupted dosing will be documented. Subjects unable to ingest the full amount of the initial [redacted] of study drug suspension or who vomit within 24 h after dosing will be withdrawn from the study and replaced (See Section 5.3.3 and 5.3.4). Subjects who do not ingest the content of the second [redacted] study drug suspension will not be withdrawn from the study.

6.6 Prior and Concomitant Medications/Treatments

Medications include the following: prescription drugs, birth control hormonal preparations, non-prescription medication, herbs, vitamins, nutritional supplements, and illicit and recreational

substances. Medications taken before or after dosing will be reported as Prior Medications or Concomitant Medications (ConMeds), respectively.

Prior prescription medication information will be recorded at Screening Visit and, except for hormonal contraceptives, should not be taken for 30 days before dosing or during the study period.

Zoliflodacin should not be co-administered in subjects using strong (i.e. avasimibe, carbamazepine, phenytoin, rifampin, and St. John's wort) or moderate (i.e. efavirenz, bosentan, etravirine, modafinil, and nafcillin) inducers of CYP3A4. These drugs should not be taken for 30 days before dosing and during the study period.

A vaccine should not be received within 30 days before dosing or before the end of the trial, except for licensed influenza vaccine during the flu season, which may be administered up to 7 days before or after dosing.

Non-prescription medications, herbs, vitamins, and nutritional supplements should not be taken within 15 days before dosing. OTC medications taken for a brief period (<48 h) for the treatment of common symptoms (such as headache, indigestion, muscle pain) may be allowed as approved by the designated study clinician.

Allowed medications include: oral contraceptives; H1 antihistamines; topical/ intranasal corticosteroids; nonsteroidal anti-inflammatory drugs (NSAIDS); licensed influenza vaccine during the flu season, 7 days before or after dosing.

Blood/blood products (RBCs, WBCs, platelets, and plasma) should not be donated within 60 days of dosing or received before Final Visit.

Following dosing, each new concomitant medication and changes to existing medications will be recorded. Subjects will be required not to utilize non-study medication during the trial except those deemed necessary by the Site PI or sub-investigator.

Any drug (e.g., non-prescription medications, herbal supplements, vitamins, or prescription medications) or vaccine or blood/blood products used by the subject during the trial will be recorded in the subject’s source documents and on the appropriate electronic case report form (eCRF), and the PI or authorized study clinician (listed on FDA Form 1572) will note whether the use was medically indicated and immediately necessary. Any use of medications not authorized by the study PI or authorized clinician will be recorded as a deviation.

6.7 Subject Restrictions

6.7.1 Physical Activity

Subjects will be asked to refrain from rigorous physical activity 15 days before dosing and during the trial.

6.7.2 Fluid and Food intake

Subjects will be provided food and non-alcoholic beverages by the CTU during the inpatient period of the trial. Subjects will fast for at least 8 h before dosing and 4 h after dosing. For clinical laboratory blood draws, subjects will fast for at least 4 h. Otherwise, there will be no restrictions regarding food and fluid intake.

6.7.3 Alcohol, Marijuana and Illicit Drugs

Alcohol should not be used at least two days before administration of study drug and during the trial.

Marijuana and illicit drugs are exclusionary if detected before dosing and prohibited during the trial.

6.7.4 Other Restrictions

Smoking or use of tobacco or nicotine-containing products is prohibited within 15 days before dosing and during the trial. Caffeinated beverages or foods containing caffeine are prohibited within 72 h before dosing and during the trial.

7 STUDY SCHEDULE

The Schedule of Study Procedures and Evaluations is included as Appendix A.

7.1 Recruitment

The subject population will be recruited from the local to the site surrounding area utilizing the CTU subject database and IRB-approved advertisements and social media. IRB-approved, prescreen questionnaires will be used to determine if subjects meet study requirements before scheduling screening visits.

7.2 Screening Visit (Day -21 to Day -2)

The following will be done within 21 days before study drug dosing:

- Obtain informed consent
- Assign a study ID number to subjects who consent to participate
- Review inclusion/exclusion criteria to ensure the subject is eligible for enrollment
- Record demographics including age, gender, race, and ethnicity
- Obtain contact information
- Obtain MH
- Review history of prior medications, including all taken within 30 days
- Perform complete PE (except genital, rectal, and breast exams) by licensed clinician listed on Form FDA 1572
- Obtain height and weight, and calculate BMI (wt [kg] / ht [m^2])
- Take VS (supine systolic and diastolic BP, HR, respiratory rate, and oral temperature)
- Obtain blood and urine samples for clinical laboratory tests
- Obtain blood samples for viral serology
- Obtain serum for β-HCG pregnancy test from all women
- Obtain serum for FSH level from only post-menopausal women
- Obtain urine sample for toxicology
- Perform breathalyzer test for alcohol use
- Obtain a 12-lead ECG with 10-sec rhythm strip
- Counsel on the avoidance of pregnancy for women of childbearing potential
- Counsel both male and females on the use of contraception
- Counsel on the avoidance of alcohol, marijuana, illicit drugs, and prohibited medications

Subjects who meet the eligibility criteria will be contacted by site personnel and asked to return to the CTU for Day -1 assessments and possible inpatient admission.

Subjects who fail screening due to a medical condition or abnormal laboratory tests including pregnancy test and positive tests for hepatitis B, hepatitis C, and HIV will be informed of the findings and counseled to seek medical care for further evaluation and treatment. If subjects test positive for HIV, HBsAg, and/or HCV infection, they will be informed that test results may be reported to the local Health Authorities according to State or Local Law.

7.3 Enrollment/Baseline

7.3.1 Admission Visit (Day -1, Unit Check-in)

Subjects meeting all of the inclusion and none of the exclusion criteria at Screening Visit will check into the CTU on Day -1 and the following procedures will be performed:

- Review inclusion/exclusion criteria to ensure the subject remains eligible for enrollment
- Update MH
- Update Prior Medications
- Perform abbreviated PE (An abbreviated complete PE will be performed if the Screening PE was completed >7 days from dosing)
- Obtain VS
- Obtain weight
- Obtain blood and urine samples for clinical laboratory tests (if screening samples were collected >7days from dosing)
- For all women, a serum β-HCG pregnancy test will be done, and negative results confirmed before dosing
- Obtain urine sample for toxicology
- Perform breathalyzer test for alcohol use
- Obtain 12-lead ECG with 10-sec rhythm strip

Subjects who meet the eligibility criteria on Day -1 will be admitted to the CTU on the same day. The subject will fast at least 8 h before dosing, but will have access to water during that period.

7.4 Inpatient period (Days 1 to 4)

7.4.1 Administration of Study Drug (Day 1)

A subject will be enrolled in the trial and receive the study drug on Day 1 if he/she continues meeting the eligibility criteria by assessments performed before dosing

Before Dosing:

- Withhold breakfast but allow access to water
- Update MH
- Update Prior Medications
- Obtain VS (within 60 min before dosing)
- Perform symptom-directed PE (if applicable)
- Obtain 12-lead ECG with 10-sec rhythm strip (within 60 min before dosing)
- May insert IV catheter for blood collection into a forearm vein
- Obtain blood (plasma) PK sample (30 min before dosing)

Dosing:

- Administer a single 4-g dose of zoliflodacin orally as a [redacted] followed by [redacted] chase
- Withhold food until 4 h after dosing but allow access to water

After Dosing:

- Obtain VS at 1 h (±10 min), 2 h (±10 min), and 4 h (±10 min) after dosing; more frequent monitoring will be at the PI's discretion based on subject's clinical presentation
- Perform symptom-directed PE (if applicable)
- Perform AE and SAE assessments
- Obtain 12-lead ECG with 10-sec rhythm strip at 1 h (±10 min), 2 h (±10 min), and 4 h (±10 min) after dosing
- Obtain blood (plasma) PK samples at 0.5 h (±5 min), 1 h (±5 min), 2 h (±5 min), 3 h (±10 min), 4 h (±10 min), 6 h (±10 min), 8 h (±15 min), and 12 h (±15 min) after dosing
- Document ConMeds

7.4.2 In-patient Follow-up Day 2

- Update MH
- Obtain VS
- Perform symptom-directed PE (if applicable)
- Perform AE and SAE assessments
- Obtain blood (plasma) PK samples at 24 h (±2 h) and 36 h (±2 h) after dosing
- Document ConMeds

7.4.3 In-patient Follow-up Day 3

- Update MH
- Obtain VS
- Perform symptom-directed PE (if applicable)

- Perform AE and SAE assessments
- Obtain blood (plasma) PK samples at 48 h (±2 h) after dosing
- Document ConMeds

7.4.4 Discharge from Unit (Day 4)

The subject is eligible for discharge from the CTU at 72 h (±2 h) after dosing, after the following assessments have been performed, and if no clinically important abnormalities are confirmed by the study PI or designated physician:

- Update MH
- Obtain VS
- Perform abbreviated PE
- Perform AE and SAE assessments
- Document ConMeds
- Obtain 12-lead ECG with 10-sec rhythm strip at 72 h (±2 h) after dosing
- Obtain blood (plasma) PK samples at 72 h (±2 h) after dosing
- Obtain blood and urine for clinical laboratory tests
- Counsel women of childbearing potential on the avoidance of pregnancy
- Counsel male and female subjects on the avoidance of pregnancy
- Remind subjects to abstain from prohibited medications, alcohol, marijuana and illegal drugs
- Instruct on the next scheduled visit
- Discharge subject from the CTU after review of clinical laboratory tests, ECG, and other assessments by PI or authorized clinician

7.5 Final Study Visit (Day 8 ± 2)

- Update MH
- Obtain VS
- Perform complete PE
- Obtain weight
- Collect blood for serum β-HCG pregnancy test in all females
- Perform AE and SAE assessments
- Update ConMeds
- Counsel male and female subjects on the avoidance of pregnancy
- Counsel female subjects to use appropriate contraception for 30 days after the final visit.
- Counsel male subjects to use appropriate contraception and refrain from sperm donation for 90 days after the final visit.

- Discharge from trial after review of clinical laboratory tests, ECG, and other assessments by PI or authorized clinician

7.6 Early Termination Visit (if needed)

- Update MH
- Obtain VS
- Perform complete PE
- Perform AE and SAE assessments
- Update ConMeds
- Obtain 12-lead ECG with 10-sec rhythm strip
- Obtain blood and urine for clinical laboratory tests
- Collect blood for serum HCG pregnancy test in all females
- Obtain blood PK sample
- Counsel male and female subjects on the avoidance of pregnancy
- Counsel female subjects to use appropriate contraception for 30 days after the ET visit.
- Counsel male subjects to use appropriate contraception and refrain from sperm donation for 90 days after the ET visit.

7.7 Unscheduled Visit (if needed)

A subject may return to the clinic for an unscheduled visit at any time. The following activities at a minimum should be performed:

- Update MH
- Obtain VS
- Perform symptom-directed PE (if applicable)
- Update ConMeds
- Perform AE and SAE assessments
- Obtain blood and/or urine for clinical laboratory tests (if applicable)
- Counsel male and female subjects on the avoidance of pregnancy

8 STUDY PROCEDURES/EVALUATIONS

8.1 Clinical Procedures/Evaluations

8.1.1 Informed Consent

The ICF must be approved by the reviewing IRB/EC and executed before performing any study-related activities.

Informed consent will be obtained for all subjects participating in the trial before performing any screening assessments. Subjects may withdraw consent at any time. Participation in the trial may be terminated at any time without the subject's consent as determined by the investigator.

8.1.2 Demographics

Demographic information (date of birth, gender, ethnicity, and race) will be recorded on the subject's source documents and eCRF at Screening Visit. Name, address, phone number, and emergency contact information will be documented in the source documents only.

8.1.3 Inclusion/Exclusion Criteria

Eligibility screening of healthy subjects will be completed within 21 days before study drug dosing and will be documented on the subject's source documents and on the eCRF. Confirmation of eligibility will be performed before dosing on Day 1.

Screening failures and the reason for failure to meet the study eligibility requirements will be documented in the source documents and entered into the study database.

8.1.4 Medical History

For subjects enrolled in the trial, the MH will be obtained by direct interview from the subject and recorded on the subject's source document and eCRF. The MH will capture the subject's current disease processes, past disease processes, history of hospitalization, history of surgery, allergies, and prior medications (taken 30 days before dosing). Subjects will be queried regarding a history of significant medical disorders of the head, eyes, ears, nose, throat (HEENT), mouth, skin, and the cardiovascular, gastrointestinal, renal, urological, nervous, hematological, lymphatic, endocrine, musculoskeletal, and genital/reproductive systems. A history of cancer, autoimmune disease, immunodeficiency, psychiatric illness, and substance abuse will be specifically solicited. The MH will be obtained at Screening Visit and updated upon admittance to the CTU and before dosing on Day 1. After the study treatment, an interim MH will be obtained on all study days by interview of subjects or by evaluating any reported symptoms, noting any changes since the previous study day or contact. Any worsening of pre-dosing MH or new symptoms will be evaluated and reported as AEs.

8.1.5 Physical Examination

A complete PE – except genital, breast, and rectal exams – will be performed at Screening Visit and Final Visit (Day 8 ± 2), or ET, and will assess general appearance, HEENT, heart, lungs, abdomen, skin, musculoskeletal system, and lymph nodes, and include an abbreviated neurological exam.

An abbreviated PE will be performed on Day -1 and Day 4. If a complete PE was performed ≤7 days before dosing, then an abbreviated PE will not be performed on Day -1. An abbreviated PE differs from a complete PE in that the abdomen and neurological system are not evaluated.

A symptom-directed PE will be performed before dosing on Day 1 and after dosing on Days 1, 2, and 3, to evaluate new symptoms or treatment-emergent AEs, respectively.

Height and weight will be measured, and BMI calculated, at Screening Visit; only weight will be measured on Day -1 and at Final Visit (Day 8 ± 2).

Refer to the protocol-specific MOP for further details. The findings of each examination will be recorded on the subject's source documents and eCRF. Any new findings on examination or worsening of existing conditions after dosing are to be reported as AEs.

8.1.6 Vital Signs

VS including resting (measured after supine for at least 10 min) systolic and diastolic BP, HR, respiratory rate, and oral temperature will be measured at Screening Visit, on Day -1, before and after dosing on Day 1, on Days 2, 3, and 4, and at Final Visit, or ET. On Day 1, VS will be measured approximately 1 h before dosing, and 1 h (±10 min), 2 h (±10 min), and 4 h (±10 min) after dosing. On Days 2, 3, and 4, VS will be measured at 24 h (±2 h), 48 h (±2 h), and 72 h (±2 h), respectively. Acceptable ranges are shown in Appendix B.

VS that are considered aberrant due to an error in measurement may be repeated. During screening and after dosing, an abnormal VS measurement may be repeated twice more at rest, within 5 min of each other. If the second measurement is abnormal, it will be reported at the highest grade of the two measurements and the subject will be excluded (if at Screening Visit) or the event reported as an AE (if after dosing). If the second measurement is normal, a third measurement will be taken at least after 5 min at rest. If the third measurement is still normal, the subject is eligible (if at Screening Visit) or there is no AE (if after dosing); if it is abnormal, the subject will be excluded (if at Screening Visit) or an AE will be reported at the highest assessed grade (between first and third measurements) and graded for severity per Appendix C (if after dosing).

8.1.7 Electrocardiogram

A 12-lead ECG and 10-sec rhythm strip will be obtained at Screening Visit and on Days -1, 1, and 4, or ET. On Day 1, a 12-lead ECG and 10-sec rhythm strip will be recorded within 1 h before dosing and 1 h (±10 min), 2 h (±10 min), and 4 h (±10 min) after dosing. The ECGs will be reviewed by the PI or a designated clinician (listed in FDA Form 1572). ECGs will be performed after the subject rests quietly in a supine position for at least 5 min. To be eligible for participation, the QT/QTcF interval must be normal, and there must be no clinically significant ECG abnormalities. If a question regarding ECG interpretation arises, the study investigators will have the ECG reviewed by a cardiologist.

8.2 Laboratory Evaluations

Venipuncture schedule and blood volumes are shown in Appendix A and Appendix D. The blood volume for hematology and chemistry tests will be approximately 9 mL per sample (Appendix D).

8.2.1 Clinical Laboratory Evaluations

8.2.1.1 Screening and Safety Clinical Laboratory (Hematology, Chemistry, and Urinalysis) Tests

Blood and urine samples for clinical laboratory tests will be collected at Screening Visit, and on Days -1 (if not collected ≤7 days of dosing) and 4, or ET. These tests will include:

- Hematology (HEM): hemoglobin (Hgb), hematocrit (Hct), red blood cell (RBC) count, platelet count, and white blood cell (WBC) count with absolute differential count
- Chemistry (CHEM): serum creatinine, with estimation of GFR, blood urea nitrogen (BUN), glucose (fasting at least 4 h), total bilirubin, direct bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (AP), total protein, albumin, sodium, chloride, potassium, bicarbonate (CO_2), magnesium
- Urinalysis (UA): Routine dipstick testing of clean-catch urine for blood, protein, and glucose; other coincidental tests will be recorded

Clinical laboratory tests at Screening Visit and Day -1 should be in the normal reference range with acceptable exceptions, as shown below and in Appendix B:

Elevated bilirubin due to documented Gilbert's syndrome that is Grade 1 is allowable*, but Grade 2 or higher is exclusionary. (To document a subject has Gilbert syndrome, a diagnosis from the medical record must be provided or the PI may make a 'presumptive' diagnosis of Gilbert syndrome in subjects with unconjugated hyperbilirubinemia on repeated testing (at least 2 samples separated in time) who have otherwise normal serum ALT/AST and AP concentrations, and a normal CBC.)*

Elevated serum glucose, sodium, potassium, bicarbonate (CO_2), total protein, and AP with a toxicity grade *of 1 is allowable.*

Serum chloride and albumin above the upper limit of normal (ULN) is allowable.

If UA by dipstick is not within acceptable ranges per Appendix B for blood, protein, or glucose, a complete UA with microscopic evaluation will be performed and the results will supersede those of the dipstick. Menstruating females failing inclusion criteria due to a positive urine dipstick or microscopic UA may be retested following cessation of menses. Do not exclude subjects with ≤6 RBC/HPF.

Laboratory *values that are outside the range of eligibility but are thought to be due to an acute condition or due to collection or laboratory error may be repeated once.*

Laboratory values will be transferred in AdvantageEDC[SM].

Abnormal safety laboratory values after dosing will be graded per the Toxicity Table, Appendix C. Abnormal values within the acceptable range per Appendix B noted at screening or baseline will only be considered AEs if they deteriorate after study drug dosing.

8.2.1.2 Viral Serology Testing

Serological testing for HIV antibody, HCV antibody, and HBsAg will be performed at Screening Visit. These tests must be negative for study eligibility. In cases where a false-positive result is suspected, confirmatory testing (e.g., Polymerase Chain Reaction) may be performed.

8.2.1.3 Pregnancy Testing

In all women, a serum β-HCG level will be measured at Screening Visit and on Day -1; results must be negative for entry into the trial. Serum pregnancy test will also be performed at Final Visit, or ET.

8.2.1.4 Serum FSH testing

A serum FSH level for confirmation of post-menopausal status in female subjects will be measured at Screening Visit only.

8.2.1.5 Urine Toxicology Screening

A urine toxicology screen will be performed at Screening Visit and on Day -1 to detect the presence of amphetamines, cocaine, barbiturates, benzodiazepines, opiates, tetrahydrocannabinol, MDMA, methadone, TCA, oxycodone, and phencyclidine. Results must be negative for study eligibility. Urine creatinine will be measured as part of the profile to assess quality of collected sample.

8.2.1.6 Alcohol testing by breathalyzer

Detection of recent alcohol consumption by breathalyzer will be performed at Screening Visit and on Day -1. Results must be negative for study eligibility.

8.2.2 Special Assays or Procedures

8.2.2.1 Pharmacokinetics Assay of Zoliflodacin

Blood (plasma) samples will be collected in K_2EDTA tubes at the following study days and time points: On Day 1 at 30 min before dosing, and 0.5 h (±5 min), 1 h (±5 min), 2 h (±5 min), 3 h (±10 min), 4 h (±10 min), 6 h (±10 min), 8 h (±15 min), and 12 h (±15 min) after dosing; on Day 2 at 24 h (± 2 h) and 36 h (± 2 h) after dosing; on Day 3 at 48 h (±2 h) after dosing; and on Day 4 at 72 h (±2 h) after dosing, or ET.

Sample collections will be scheduled for the nominal time point and actual collection times recorded in the source documents.

Plasma concentrations of zoliflodacin will be determined using a validated HPLC-MS/MS method.

8.2.3 Specimen Preparation, Handling, and Shipping

8.2.3.1 Instructions for Specimens Preparation, Handling, and Storage

Details regarding the specimen preparation, handling, and storage are described in the protocol-specific MOP.

8.2.3.2 Specimen Shipment

Specimen shipment will occur at intervals during the trial following all applicable International Air Transport Association (IATA) requirements and according to the specifics for storage temperature and documentation as detailed in the central (clinical) laboratory manual and protocol-specific MOP, as appropriate.

All specimens for clinical screening and safety laboratory evaluations will be transported from the CTU to the local clinical laboratory.

Plasma samples for bioanalytical assays will be shipped from the CTU to the DMID-CMS (Fisher BioServices) at:

Fisher BioServices

c/o DMID Clinical Materials Services (CMS)
20439 Seneca Meadows Parkway
Germantown, MD 20876
Phone: 240-477-1350
Fax: 240-477-1360
Email: DMID.CMS@thermofisher.com

The plasma samples will then be provided by DMID-CMS to the bioanalytical lab, KCAS Inc., at:

KCAS Bioanalytical Services
12400 Shawnee Mission Parkway
Shawnee, KS 66216
POC: Marsha Luna
Senior Manager, PBI
Office: 913-248-3042
Email: marsha.luna@kcasbio.com

9 ASSESSMENT OF SAFETY

Regulatory requirements including FDA regulations and ICH Guidelines for GCP set forth safety monitoring and reporting responsibilities of sponsors and investigators to ensure the safety and protection of human subjects participating in clinical trials.

Responsibilities:

Investigators participating in this clinical trial are responsible for and will:

- Evaluate subject safety including assessment of AEs for seriousness, severity, and causality (relatedness to study drug);
- Notify the sponsor (DMID) of SAEs within 24 h of site awareness;
- Provide detailed written reports, including necessary documentation requested by the sponsor or IRB/EC, promptly following immediate initial reports;
- Inform the IRB/EC of SAEs and AEs as required by applicable regulatory requirements.

9.1 Specification of Safety Parameters

Safety will be assessed by the timing, frequency, relatedness to study drug, and severity of:

1. Treatment-emergent SAEs occurring from dosing through Final Visit (Day 8 ± 2), or ET;
2. Clinical laboratory AEs occurring from dosing through Final Visit (Day 8 ± 2), or ET;
3. Non-serious, unsolicited treatment-emergent AEs occurring from dosing through Final Visit (Day 8 ± 2), or ET.

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

9.2.1 Adverse Events

Definitions

Adverse events

ICH E6 defines an AE as any untoward medical occurrence in a subject or clinical investigation subject who was administered a pharmaceutical product, regardless of its causal relationship to the product. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with use of the product. The occurrence of an AE may come to the attention of study personnel during study visits and interviews, or by a subject presenting for medical care.

The FDA defines an AE as any untoward medical occurrence associated with the use of a drug in humans, whether it is considered drug-related or not.

Any medical condition that is present at the time that the subject is screened should be considered as baseline and not reported as an AE. However, if the condition increases in severity or frequency at any time during the trial, it should be recorded as an AE.

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

9.2.1.1 Severity of Events

Intensity of AEs will be graded as follows, unless otherwise specified in Appendix C:

Mild: Require minimal or no treatment; do not interfere with the subject's daily activities.

Moderate: Result in a low level of inconvenience or concern with therapeutic measures; may cause some interference with functioning.

Severe: Interrupt a subject's usual daily activity and may require systemic drug therapy or other treatment; are usually incapacitating.

9.2.1.2 Relationship to Study Products

AEs and SAEs must be assessed by the investigator to determine relationship to the study drug, using the following two terms. In a clinical trial, the study drug must always be suspect.

- **Related:** There is a reasonable possibility that the study drug caused the AE/SAE; that is, there is evidence to suggest a causal relationship
- **Not Related**: There is not a reasonable possibility that the study drug caused the AE/SAE

The investigator must provide an assessment of association or relationship of each AE/SAE to the study drug based on:

- Temporal relationship of the AE/SAE to study drug dosing;
- Whether an alternative etiology has been identified;
- Biological plausibility;
- Existing therapy and/or ConMeds.

9.2.1.3 Reporting Adverse Events

AEs will be captured on the appropriate subject's source document and eCRF. Information collected for AEs includes event description, time of onset, investigator assessment of severity, relationship to the study drug, date of resolution of the event, seriousness, and outcome.

All AEs will be documented from the time of drug dosing up to and including Final Visit. AEs will be followed to resolution or until considered stable in the clinical judgment of the study investigator. Evaluation of AEs may require unscheduled visits and clinical and laboratory investigations, according to the clinical judgment of the Site PI and study physicians.

9.2.2 Reactogenicity (for Vaccine Studies and Some Therapeutic Trials)

Not applicable.

9.2.3 Serious Adverse Events

An SAE is any AE that meets at least one of the following criteria:

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

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

SAEs will be:

- Assessed for severity and relationship to study product and alternate etiology by an authorized study physician (listed on FDA Form 1572);
- Recorded on the appropriate SAE data collection form and eCRF;
- Followed through resolution;
- Reviewed and evaluated by an ISM (as deemed necessary), the SMC (periodic review unless related), DMID, and the IRB.

9.2.4 Procedures to be Followed in the Event of Abnormal Laboratory Test Values or Abnormal Clinical Findings

A licensed study clinician (listed on FDA Form 1572) will make the determination of seriousness, severity, and causality; provide a medical evaluation of AEs; and classify AEs based upon medical judgment.

Abnormal laboratory values or clinical findings for all enrolled subjects, including values and findings noted at screening and baseline, will be assessed using the toxicity scales in Appendix C. Abnormal values and findings noted at screening or baseline will only be considered AEs if

they deteriorate after study drug dosing. For abnormalities noted from the time of study drug dosing, any Grade 1 or higher laboratory abnormality listed on the toxicity table in Appendix C will be entered in the database as an AE. Safety laboratory results that are abnormal according to the local laboratory reference range, but not considered a Grade 1 abnormality, will be evaluated by the study site clinician and reported as Grade 1 abnormality if clinically significant. If not clinically significant, these will not be considered laboratory AEs and will thus not be graded, but will be recorded in the source document and followed-up clinically at the discretion of the study site clinician. Abnormal laboratory values, performed as part of the complete CBC, clinical chemistry or UA but not listed in this toxicity table will be evaluated by the study clinicians, recorded in the source document and, if clinically significant, considered AEs and graded according to the criteria in Section 9.2.1.

Protocol-specific laboratory normal range values in effect at the time of protocol submission are included in Appendix B. Changes in these ranges during the trial will be handled as administrative protocol amendments and toxicity grades will be calculated using the new ranges from the effective date of the change. A regular protocol amendment will be submitted for these changes if a revised laboratory normal range value meets criteria for Grade 2 toxicity.

Gross blood in urine that is confirmed due to a menstrual cycle is not an AE (but is for all other reasons).

9.3 Reporting Procedures

9.3.1 Serious Adverse Events

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

All SAEs will be:

- Recorded on the appropriate SAE report form and sent to DMID Pharmacovigilance Group (PVG)
- Entered into the appropriate subject source document and eCRF in AdvantageEDC^{SM}
- Reported to the Site ISM and the IRB
- Reviewed and followed to resolution or stability by an authorized study physician (listed on FDA Form 1572)
- Collected on each subject until Final Visit

Any AE that meets a protocol-defined serious criterion must be submitted immediately (within 24 h of site awareness) on an SAE report form to DMID PVG:

DMID Pharmacovigilance Group
Clinical Research Operations and Management Support (CROMS)
6500 Rock Spring Drive, Suite 650
Bethesda, MD 20817, USA
SAE Hot Line: 1-800-537-9979 (US) or 1-301-897-1709 (outside US)
SAE FAX Number: 1-800-275-7619 (US) or 1-301-897-1710 (outside US)
SAE Email Address: PVG@dmidcroms.com

In addition to the SAE report form, selected SAE data fields must also be entered into AdvantageEDC[SM]. Please see the protocol-specific MOP for details regarding this procedure.

Other supporting documentation of the SAE may be requested by DMID PVG and should be provided as soon as possible.

The site will copy the ISM on SAE reports provided to the DMID PVG. The DMID MM and DMID CPM will be notified of the SAE by the DMID PVG. The DMID MM will review and assess the SAE for regulatory reporting and potential impact on study subject safety and protocol conduct.

At any time after completion of the study, if the site PI or appropriate sub-investigator becomes aware of an SAE that is suspected to be related to study product, the site PI or appropriate sub-investigator will report the event to the DMID PVG.

9.3.2 Regulatory Reporting for Studies Conducted Under DMID Sponsored IND

Following notification from the investigator, DMID, the Investigational New Drug (IND) sponsor, will report any suspected AE that is both serious and unexpected. DMID will report an AE as a suspected AE only if there is evidence to suggest a causal relationship between the drug and the AE. DMID will notify FDA and all participating investigators (i.e., all investigators to whom the sponsor is providing drug under its IND(s) or under any PI's IND(s)) in an IND safety report of potential serious risks from clinical trials or any other source, as soon as possible, but in no case later than 15 calendar days after the sponsor determines that the information qualifies for reporting as specified in 21 CFR Part 312.32. DMID will also notify FDA of any unexpected fatal or life-threatening suspected AE as soon as possible, but in no case later than 7 calendar days after the sponsor's initial receipt of the information. Relevant follow up information to an IND safety report will be submitted as soon as the information is available. Upon request from FDA, DMID will submit to FDA any additional data or information that the agency deems necessary, as soon as possible, but in no case later than 15 calendar days after receiving the request.

All serious events designated as “not related” to the study drug, will be reported to the FDA at least annually in a summary format.

9.3.3 Other Adverse Events (if applicable)

9.3.3.1 Reporting of Overdose

An overdose is defined as a dose greater than the high-dose level evaluated in the trial as described in Section 6.3.1 of the protocol. All overdoses should be reported; if the overdose is associated with an AE, then the AE should also be reported. In the event of an overdose of study drug, the investigator should use clinical judgment in treating the overdose and contact the DMID MM. The investigator should refer to the relevant document(s) for detailed information regarding warnings, precautions, contraindications, AEs, and other significant data pertaining to zoliflodacin. Such documentation may include but not be limited to the IB.

9.3.4 Reporting of Pregnancy

Pregnancies that occur in female subjects during the study period will be reported via AdvantageEDC^{SM} on a pregnancy report form. With the subject’s permission, all protocol-required venous blood samples will be obtained and the subject will continue to be followed for safety until Final Visit. Efforts will be made to follow all pregnancies reported during the trial to pregnancy outcome, as described in the protocol-specific MOP (e.g., delivery, spontaneous abortion, or therapeutic abortion), pending the subject’s permission.

A female subject who participates in the trial and becomes pregnant will be asked to inform study personnel of a pregnancy occurring 30 days after the final study visit. A male subject who participates in the trial and whose female partner becomes pregnant 90 days after the final study visit will be asked to inform study personnel of the pregnancy. For all reported pregnancies, subjects will be asked to provide pregnancy outcome upon delivery or pregnancy termination to the CTU.

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

Treatment-emergent SAEs and AEs will be followed until resolution or until considered stable in the clinical judgment of the investigator. Resolution of an AE is defined as the return to pretreatment status or stabilization of the condition in the clinical judgment of the study investigator, with the expectation that it will remain chronic.

Follow-up procedures, evaluations, and outcomes will be recorded in the subject's source document and eCRF.

9.5 Halting Rules

9.5.1 Study Halting Criteria

Study dosing must be stopped and a review of available safety data will be conducted by the SMC if any of the following occur on Day 1 after dosing:

1. Death of a subject following dosing regardless of relatedness to study drug;
2. One subject with an SAE that is considered related to study drug by the study PI or designated physician;
3. Two or more subjects with a Grade 3 systemic AE coded in the same organ system that is considered related to the study drug by the study PI or authorized clinician (listed in FDA Form 1572);

   Note: VS abnormalities should be considered part of a systemic disorder or an organ-specific condition, as described in Appendix C, in order to be included among the Study Halting Criteria.
4. An overall pattern of symptomatic, clinical non-serious events that the study PI or authorized clinician considers associated with study drug and that may appear minor in terms of individual events, but that may collectively represent a serious potential safety concern and pose a risk to continuing the subject in the trial.

9.6 Safety Oversight (ISM plus SMC)

9.6.1 Independent Safety Monitor (ISM)

The ISM is a physician with relevant expertise whose primary responsibility is to provide independent safety monitoring in a timely fashion. The ISM will review SAEs and other AEs as needed and provide an independent assessment to DMID.

9.6.2 Safety Monitoring Committee (SMC)

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

The SMC will meet as follows:

- Organizational meeting (before study initiation)
- *Ad hoc* meeting
  - When study halting criteria are met
  - At the request of DMID to review a potential safety concern identified by either the Site PI, ISM, or DMID MM

- Scheduled meeting
  - The SMC will review all available safety data when available

Procedures for SMC reviews/meetings will be defined in the SMC charter. The SMC will review safety data and advise on using the 4-g dose of zoliflodacin in a subsequent thorough QT study.

10 CLINICAL MONITORING

10.1 Site Monitoring Plan

Site monitoring is conducted to ensure that human subject protections, study and laboratory procedures, study intervention administration, and data collection processes are of high quality and meet sponsor, GCP/ICH, and regulatory guidelines, when appropriate. Site visits may be conducted by an authorized representative of DMID or other regulatory agencies to inspect study data, subjects' medical records, and eCRFs in accordance with ICH guidelines, GCP, and the respective local and national government regulations and guidelines.

The investigator will permit authorized representatives of DMID and the respective local and national health authorities to inspect facilities and records relevant to the trial, if needed.

A separate monitoring plan developed by DMID will describe protocol-specific items to be monitored.

Site visits will be made at standard intervals defined by DMID and may be made more frequently as directed by DMID. Monitoring visits will include, but not be limited to, review of regulatory files, accountability records, subject's source documents, eCRFs, ICFs, clinical and laboratory reports, and protocol and GCP compliance. Site monitors will have access to each participating site, study personnel, and all study documentation according to the DMID-approved site monitoring plan. Study monitors will meet with the Site PI to discuss any problems and actions to be taken and document visit findings and discussions.

11 STATISTICAL CONSIDERATIONS

11.1 Study Hypotheses

There are no formal hypotheses being tested in this Phase 1 trial. The objectives of the trial are to obtain additional PK and safety data for a single 4-g oral dose of zoliflodacin.

11.1.1 Study Objectives and Outcome Measures

11.1.1.1 Study Objectives

Primary Objective: To evaluate the plasma PK of a single 4-g oral dose of zoliflodacin under fasting conditions

Secondary Objective: To evaluate the safety and tolerability of a single 4-g oral dose of zoliflodacin

11.1.1.2 Study Outcome Measures

Primary Endpoints: C_{max}, T_{max}, $AUC_{(0\text{-last})}$, and other PK parameters determined using plasma concentrations of zoliflodacin in blood samples collected on Day 1 [baseline (30 min before dosing) and 0.5 h, 1 h , 2 h , 3 h , 4 h , 6 h , 8 h , and 12 h after dosing], Day 2 [24 h and 36 h after dosing], Day 3 [48 h after dosing], and Day 4 [72 h after dosing], or ET, and measured using a validated HPLC-MS/MS method

Secondary Endpoints:

- The occurrence of treatment-emergent SAEs from administration of zoliflodacin on Day 1 to Final Visit (Day 8 ± 2), or ET
- The occurrence of unsolicited AEs from administration of zoliflodacin on Day 1 to Final Visit (Day 8 ± 2), or ET
- The changes from baseline (up to 60 min before dosing) in VS following administration of zoliflodacin as measured on Day 1 [1 h, 2 h, and 4 h after dosing], Days 2, 3 and 4 [24 h, 48 h, and 72 h, respectively, after dosing], and Final Visit (Day 8 ± 2) , or ET
- The changes from baseline (tested on Day-1) in clinical laboratory test values following administration of zoliflodacin, as measured on Day 4 [72 h after dosing], or ET
- The changes from baseline (up to 60 min before dosing) in ECG parameters following administration of zoliflodacin, as measured on Day 1 [1 h, 2 h, and 4 h after dosing] and Day 4 [72 h after dosing], or ET

11.2 Sample Size Considerations

Since this is a pilot Phase 1, open-label trial with a single 4-g dose cohort of zoliflodacin, no formal sample size calculations based on testing a statistical hypothesis were constructed. Eight subjects will be enrolled and it is planned to have seven evaluable subjects given zoliflodacin. This sample size will provide sufficient information to estimate exposure to the 4-g dose of zoliflodacin and to assess its safety.

11.3 Safety Review

An SMC will be appointed to oversee the safe conduct of the trial. A scheduled SMC meeting will be held after all subjects complete the trial to review safety data, and advice on using the 4-g dose in a subsequent thorough QTc study. If criteria for halting the trial (as listed in Section 9.5.1) are met during dosing on Day 1, an *ad hoc* SMC meeting will be held to review all available safety data and to make recommendations about the dosing of all further subjects in the trial.

11.4 Final Analysis Plan

The ICH/ FDA Guidance Document E9 (Statistical Principles for Clinical Trials) will be followed for all statistical content.[16] For categorical data, summaries of frequencies and percentages will be presented. Summaries for continuous data will include minimum, median, mean, standard deviation, and maximum. Details of study analyses for safety and PK data and their presentation will be described in the Statistical Analysis Plan (SAP) and accompanying Tables, Listings and Figures (TLF) templates. The SAP will be prepared and finalized by the DCC prior to final data lock. Analyses included in the CSR will be performed by the DCC after final data lock. Any change from originally planned statistical analyses will be reported in the final clinical study report (CSR).

11.4.1 Analysis Populations

All subjects who received the study drug will be included in the safety population and analyzed as treated. The PK analysis population will consist of all subjects who received zoliflodacin and have at least one quantifiable post-dosing drug concentration measured. The PK analysis subset will be based on the PK population, which includes all subjects who completed the PK part of the trial without any protocol violations that would likely affect the PK results and who have evaluable plasma concentration data for zoliflodacin from whom at least a subset of the designated PK parameters can be determined.

11.4.2 Demographics and Baseline Characteristics

Subject demographics and baseline characteristics will be summarized. The number of subjects who enroll in the trial, and the number and percentage of subjects who complete each assessment, will be presented. The percentage of subjects who withdraw from the trial or discontinue the study drug, and reasons for withdrawal or discontinuation, will be summarized.

11.4.3 Safety Analysis Plan

11.4.3.1 Adverse and Serious Adverse Events

AEs will be coded using the latest version of the Medical Dictionary for Regulatory Activities® (MedDRA). All AEs occurring after study drug dosing will be summarized using frequency counts and percentages. The following summaries will be presented for AEs and SAEs:

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

Unless otherwise specified, at each level of subject summarization in reporting the incidence of AEs, a subject will be counted once if the subject reported one or more AEs. If more than one occurrence of an AE is reported, the AE of the worst severity or the worst-case relationship assessment will be summarized.

11.4.3.2 Additional Safety Analyses

Descriptive summary statistics (mean, standard deviation, median, min, and max) for clinical laboratory data, ECG parameters, and VS at admission and each applicable post-dosing visit, including changes from baseline values, will be calculated. If multiple baseline values are obtained, only the most recent value will be analyzed. For change from screening summaries, subjects with an undefined change from screening, because of missing data, will be excluded. Clinical significance of abnormalities will be indicated in the listings.

11.4.4 PK Analysis Plan

PK parameters will be estimated for zoliflodacin by noncompartmental methods using version 6.4 or higher of Phoenix® WinNonlin®. When evaluable, estimated PK parameters will include:

- $AUC_{(0\text{-last})}$: 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 oral clearance
- Vz/F: Apparent volume of distribution

Other PK parameters may be calculated, as appropriate. Linearity of terminal elimination slope will be assessed statistically by linear regression, which provides estimates for the slope, intercept, and r^2 (or adjusted r^2) to calculate elimination rate constant, and helps define goodness of fit. Details will be provided in the SAP.

The results will be listed by subject, and summarized with descriptive statistics including: n, mean, standard deviation, coefficient of variation (CV %), median, minimum, maximum, geometric mean, and geometric standard deviation.

Graphical presentations of concentration vs. time profiles will be provided for zoliflodacin, and will include individual subject and mean concentration profiles. Semi-log concentration profiles will be provided for individual subjects.

11.4.5 Missing Values and Outliers

All attempts will be made to collect all data per protocol. No imputation will be performed for missing values. Outliers will not be excluded from the primary analyses. Outliers identified during the PK analysis will be discussed in the analysis report.

12 SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA/DOCUMENTS

The site will maintain appropriate medical and/or research records for the trial, in compliance with ICH E6 GCP, Section 4.9, and regulatory and institutional requirements for the protection of confidentiality of subjects. As part of participating in a DMID-sponsored, DMID-affiliated, or manufacturer-sponsored trial, the site will permit authorized representatives of the sponsor (DMID), to include Emmes (the DCC), DynPort Vaccine Company, LLC (DVC), and regulatory agencies to review (and, when required by applicable law, to copy) clinical records for the purposes of quality assurance reviews, audits, and evaluation of the study safety and progress.

Forms for use as source documents will be derived from eCRFs and will be provided by the DCC and the Site. Additional source data are all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Examples of these original documents and data records include, but are not limited to, hospital records, clinical and office charts, laboratory notes, memoranda, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, ECG print-outs, and subject files and records kept at the pharmacy, laboratories, and medico-technical departments involved in the clinical trial.

13 QUALITY CONTROL AND QUALITY ASSURANCE

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

The DCC will implement quality control procedures beginning with the data entry system and generate data quality control checks that will be run on the database. Any missing data or data anomalies will be communicated to the site(s) for clarification and resolution.

14 ETHICS/PROTECTION OF HUMAN SUBJECTS

14.1 Ethical Standard

The PI will ensure that the study is conducted in full conformity with principles of the Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research of the National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research (April 18, 1979) and codified in 45 CFR 46, 21 CFR 50 and 56, and ICH E6; 62 Federal Regulations 25692 (1997), if applicable. The PI's institution will hold a current Federal Wide Assurance (FWA) issued by the Office of Human Research Protection for federally-funded research.

14.2 Institutional Review Board

The CTU will provide for the review and approval of this protocol and the associated ICFs by an appropriate IRB/EC listed on the FWA. Any amendments to the protocol or consent materials must also be approved before they are used, unless change is for the safety of the subject. Only those IRB members who are independent of the investigators and the sponsor should provide an opinion on study-related matters. Verification of IRB approval of the protocol and the written ICF will be transmitted by the investigator or designee before shipment of the study drug. No deviations from or changes to the protocol will be initiated without prior approval of an appropriate amendment unless change is for the safety of the subject.

14.3 Informed Consent Process

The written consent document will embody the elements of informed consent as described in the Declaration of Helsinki and will adhere to the ICH Harmonized Tripartite Guideline for GCP. Informed consent should be implemented before any protocol-specified procedures or interventions are carried out. Informed consent will be obtained in accordance with 21 CFR 50.25 and 45 CFR 46. Information should be presented both orally and in written form.

An investigator or designee will describe the protocol to potential subjects face-to-face. The ICF may be read to the subjects, but, in any event, the investigator shall give the subjects ample opportunity to inquire about details of the trial and ask any questions before signing the ICF.

Study staff must inform subjects that the trial involves research, and explain the purpose of the trial, those aspects of the trial that are experimental, any expected benefits, all possible risks (including a statement that the particular treatment or procedure may involve risks to the subject or to the embryo or fetus, if the subject is or may become pregnant, that are currently unforeseeable), the expected duration of the subject's participation in the trial, the procedures of the research study, including all invasive procedures, and the probability for random assignment

to treatment groups. Subjects will be informed that they will be notified in a timely manner if information becomes available that may be relevant to their willingness to continue participation in the trial. They must also be informed of alternative procedures that may be available, and the important potential benefits and risks of these available alternative procedures. Subjects must receive an explanation as to whether any compensation and any medical treatments are available if injury occurs, and, if so, what they consist of, or where further information may be obtained. Subjects must be informed of the anticipated financial expenses, if any, to the subject for participating in the trial, as well as any anticipated prorated payments, if any, to the subject for participating in the trial. They must be informed of whom to contact (e.g., the investigator) for answers to any questions relating to the research project. Information will also include the foreseeable circumstances and/or reasons under which the subject's participation in the trial may be terminated. The subjects must be informed that participation is voluntary and that they are free to withdraw from the trial for any reason at any time without penalty or loss of benefits to which the subject is otherwise entitled.

Neither the investigator, nor the trial staff, should coerce or unduly influence a subject to participate or continue to participate in the trial. The extent of the confidentiality of the subjects' records must be defined, and subjects must be informed that applicable data protection legislation will be followed. Subjects must be informed that the monitor(s), auditor(s), IRB, NIAID, and regulatory authority(ies) will be granted direct access to the subject's original medical records for verification of clinical trial procedures and/or data without violating the confidentiality of the subject, to the extent permitted by the applicable laws and regulations, and that, by signing a written ICF, the subject is authorizing such access. Subjects must be informed that records identifying the subject will be kept confidential, and, to the extent permitted by the applicable laws and/or regulations, will not be made publicly available and, if the results of the trial are published, the subject's identity will remain confidential.

ICFs must be in a language fully comprehensible to the prospective subjects. Informed consent shall be documented using a written consent form approved by the IRB and signed and dated by the subject and the person who conducted the informed consent discussion. The signature confirms that the consent is based on information that has been provided and all questions have been answered to the prospective participant's satisfaction. Each subject's signed ICF must be kept on file by the investigator for possible inspection by regulatory authorities and/or the sponsor and regulatory compliance persons. The subject should receive a copy of the signed and dated written ICF and any other written information provided to the subjects, and should receive copies of any signed and dated ICF updates and any amendments to the written information provided to subjects.

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

Children under age 18 years will be excluded from participation because insufficient data are available in adults to judge potential risk in children, and as a Phase I trial, there is no known benefit.

Neither women nor minorities will be routinely excluded from participation in this study. Subjects will be recruited without regard to gender or race. It is expected that race and gender distributions in this study will approximate the proportion to their numbers within the community.

Women of childbearing potential will be included but will be repeatedly counseled to use effective measures (Section 5.1) to avoid becoming pregnant from the time of screening until 30 days after the last dose of study drug is received because the effect of the study drug on the unborn fetus are not known.

14.5 Subject Confidentiality

Subject confidentiality is held strictly in trust by the participating investigators, their staff, and the sponsor and their agents. This confidentiality is extended to cover testing of biological samples, and also clinical information related to participating subjects.

The study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the trial or the data will be released to any unauthorized third party without prior written approval from the sponsor. This information and data will not be used by the Site PI or other study personnel for any purpose other than conducting the trial. These restrictions do not apply to: (1) information which becomes publicly available through no fault of the Site PI or other study personnel; (2) information which is necessary to disclose in confidence to an IRB solely for the evaluation of the trial; (3) information which is necessary to disclose in order to provide appropriate medical care to a study subject; or (4) study results which may be published as described in Section 16.

The study monitors or other authorized representatives of the sponsor may inspect all documents and records required to be maintained by the Investigator, including, but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the subjects in the trial. The CTU will permit access to such records.

14.6 Study Discontinuation

DMID has the right to terminate the trial or the site's participation at any time. Reasons for terminating the trial may include, but are not limited to:

- Incidence or severity of AEs indicates a potential health hazard
- Data recording is inaccurate or incomplete

- Investigator does not adhere to the protocol or applicable regulatory guidelines in conducting the trial

If the trial is discontinued, subjects who have signed the ICF and received the study drug will continue to be followed for safety for the duration of the trial. No further study treatments will be administered to other subjects.

15 DATA HANDLING AND RECORD KEEPING

The Site PI is responsible to ensure the accuracy, completeness, legibility, and timeliness of reported data. All data collection forms should be completed legibly to ensure accurate interpretation of data. Black or blue ink is required to ensure clarity of reproduced copies. When making changes or corrections, cross out the original entry with a single line, and initial and date the change. Do not erase, overwrite, or use correction fluid or tape on the original.

Data collection forms will be provided by the DCC and the CTU and will be used by the CTU to record and maintain data for each subject enrolled in the trial. Data reported in the eCRF derived from source documents should be consistent with the source documents or the discrepancies should be explained.

DMID and/or its designee will provide guidance to investigators and other study personnel on making corrections to the data collection forms, source documents, and eCRFs.

15.1 Data Management Responsibilities

All source documents and laboratory reports must be reviewed by the clinical team and data entry staff, who will ensure that they are accurate and complete. AEs must be graded, assessed for severity and causality, and reviewed by the Site PI or designee.

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the Site PI. During the trial, the investigator must maintain complete and accurate documentation for the trial.

Emmes will serve as the DCC for the trial, and will be responsible for data management, quality review, analysis, and reporting of the study data.

15.2 Data Capture Methods

Clinical data (including, but not limited to AE/SAEs, ConMeds, MH, and PE) and clinical laboratory data will be entered into a 21CFR11-compliant Internet Data Entry System provided by the DCC. The data system includes password protection and internal quality checks (e.g., automatic range checks) to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the data collection forms completed by the study personnel.

15.3 Types of Data

Data for the trial will include clinical safety assessments, laboratory safety assessments, and PK parameters.

15.4 Timing/Reports

A final CSR will be prepared after all safety and PK data are available. See Section 9.6.2 for additional reporting requirements.

15.5 Study Records Retention

Study files ICFs must be maintained for at least 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region, or at least 2 years have elapsed since the formal discontinuation of clinical development of the study drug. These documents should be retained for a longer period, however, if required by local regulations. No records will be destroyed without the written consent of the sponsor, if applicable. It is the sponsor's responsibility to inform the investigator when these documents no longer need to be retained.

15.6 Protocol Deviations

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

These practices are consistent with ICH E6:

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

5.1 Quality Assurance and Quality Control, Section 5.1.1

5.20 Noncompliance, Sections 5.20.1, and 5.20.2.

It is the responsibility of the Site PI/study staff to use continuous vigilance to identify and report deviations within 5 working days of identification of the protocol deviation, or within 5 working days of the scheduled protocol-required activity. All deviations must be promptly reported to DMID via DCC's AdvantageEDC^{SM}.

All protocol deviations, as defined above, must be addressed in study subject source documents. A completed copy of the DMID Protocol Deviation Form must be maintained in the regulatory file, as well as in the subject's source document. Protocol deviations must be sent to the local IRB/EC per their guidelines. The Site PI/study staff is responsible for knowing and adhering to their IRB requirements.

16 PUBLICATION POLICY

Following completion of the trial, the investigator is expected to publish the results of this research in a scientific journal. The International Committee of Medical Journal Editors (ICMJE) member journals have adopted a trials-registration policy as a condition for publication. This policy requires that all clinical trials be registered in a public trials registry such as ClinicalTrials.gov*, which is sponsored by the National Library of Medicine, on or before subject enrollment. Other biomedical journals are considering adopting similar policies. It is the responsibility of DMID to register the trial in an acceptable registry.

The ICMJE defines a clinical trial as any research project that prospectively assigns human subjects to intervention or comparison groups to study the cause-and-effect relationship between a medical intervention and a health outcome. Studies designed for other purposes, such as to study PK or major toxicity (e.g., Phase I trials), are exempt from this policy.

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   De Angelis C, Drazen JM, Frizelle FA, Haug C, Hoey J, Horton R, et al. Clinical trial registration: a statement from the International Committee of Medical Journal Editors. N Engl J Med. 2004; 351:1250-1.

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2. Barbee LA, Dombrowski JC. Control of Neisseria gonorrhoeae in the era of evolving antimicrobial resistance. Infect Dis Clin North Am 2013;27(4):723-37

3. World Health Organization, Department of Reproductive Health and Research. Global incidence and prevalence of selected curable sexually transmitted infections - 2008. Available at: http://www.who.int/reproductivehealth/publications/rtis/stisestimates/en/index.html (accessed on 10 July 2014)

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5. Centers for Disease Control and Prevention (CDC). Antibiotic Resistance Threats in the United States, 2013. Available at: https://www.cdc.gov/drugresistance/threat-report-2013/pdf/ar-threats-2013-508.pdf (accessed on 10 July 2014)

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8. Investigator's Brochure. Product Name, Zoliflodacin (Research number [redacted]), [redacted]

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11. Su X-H, Wang B-X, Le W-J, et al. Multidrug-Resistant Neisseria gonorrhoeae Isolates from Nanjing, China, Are Sensitive to Killing by a Novel DNA Gyrase Inhibitor, ETX0914 (AZD0914). Antimicrob Agents Chemother. 2016 Jan; 60(1): 621–623

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16. Guidance for Industry. E9 Statistical Principles for Clinical Trials. FDA, September 1998. https://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm073137.pdf (accessed on 27 July 2017)

18 APPENDICES

APPENDIX A: SCHEDULE OF STUDY PROCEDURES AND EVALUATIONS

Study Visit	Screening [1]	Check-in	Inpatient Period				Final Visit	Unscheduled	Early termination
			Dosing	Follow Up					
Study Day / Assessments	**-21 to -2**	**-1**	**1**	**2**	**3**	**4**	**8 (± 2)**		
Informed consent	X								
Inclusion/Exclusion criteria	X	X	X						
Demographics	X								
Medical history	X								
Medical history update		X	X	X	X	X	X	X	X
Prior and ConMeds [2]	X	X	X	X	X	X	X	X	X
Complete PE [3]	X						X		X
Abbreviated PE [4]		X				X			
Symptom-directed PE [5]			X	X	X			X	
Height, weight, BMI [6]	X								
Weight		X					X		
Vital signs [7]	X	X	X	X	X	X	X	X	X
Clinical labs (HEM, CHEM, UA) [8]	X	X				X		X	X
Viral serology [9]	X								
Serum pregnancy test	X[13]	X					X		X
Serum FSH level	X[13]								
Urine toxicology	X	X							
Alcohol breathalyzer test	X	X							
12-lead ECG [10]	X	X	X			X			X
Study drug dosing			X						
PK samples [11]			X	X	X	X			X
Counsel on use of contraception and avoidance of pregnancy[14]	X					X	X		X

Study Visit	Screening [1]	Check-in	Inpatient Period				Final Visit	Unscheduled	Early termination
			Dosing	Follow Up					
Study Day / Assessments	**-21 to -2**	**-1**	**1**	**2**	**3**	**4**	**8 (± 2)**		
Counsel to avoid use of prohibited medications, alcohol, marijuana and illicit drugs	X	X				X	X		
Interim medical history			X	X	X	X	X	X	X
AE and SAE review [12]			X	X	X	X	X	X	X
Admit to CTU		X							
Discharge from CTU						X			
Discharge from trial							X		

1. Screening Visit is completed within 21 days before study drug dosing and may require more than one visit.
2. Prior medications include prescription drugs taken 30 days before dosing, and non-prescription drugs, herbs, vitamins, and nutritional supplements taken 15 days before dosing. Concomitant medications include those taken after dosing.
3. Complete PE (except genital, breast, and rectal exam): at Screening, and Final Visit, or ET.
4. Abbreviated PE: on Day -1 (not performed if the complete PE was performed ≤7 days from this visit) and Day 4.
5. Symptom-directed PE: on Days 1 (predose and postdose), 2, and 3 for evaluation of new symptoms pre-dose and AEs post-dose.
6. BMI is calculated as wt (kg) / ht (m^2).
7. Vital Signs (BP, HR, RR, T): at Screening Visit; on Days -1, 1, 2, 3, and 4; and at Final Visit, or ET. On Day 1, VS at baseline (approximately 1 h before dosing) and at 1 h (±5 min), 2 h (±10 min), and 4 h (±10 min) after dosing.
8. Clinical laboratory testing with minimum 4 h fast: HEM (Hgb, Hct, RBC, WBC with differential absolute count, platelet count); CHEM (creatinine with estimation of GFR, BUN, glucose, total bilirubin, direct bilirubin, AST, ALT, AP, total protein, albumin, electrolytes (sodium, potassium, chloride, bicarbonate (CO_2), magnesium); and dipstick UA (blood, protein, glucose): at Screening, Day -1 (if not collected ≤7 days before dosing), Day 4, or ET.
9. Viral Serology (HIV antibody, HBsAg, HCV antibody): at Screening Visit.
10. 12-lead ECG with 10 sec rhythm strip: at Screening Visit, Day -1, Day 1 [within 1 h before dosing, and at 1 h (±10 min), 2 h (±10 min), and 4 h (±10 min) after dosing], and Day 4 [72 h (±2 h)] after dosing, or ET.
11. Blood (plasma) PK samples: within 30 min before dosing, and at 0.5 h (±5 min), 1 h (±5 min), 2 h (±5 min), 3 h (±10 min), 4 h (±10 min), 6 h (±10 min), 8 h (±15 min), 12 h (±15 min), 24 h (±2 h), 36 h (± 2 h), 48 h (± 2 h), and 72 h (± 2 h) after dosing, or ET.
12. Collect all AEs from the time of dosing to and including Final Visit. Follow-up AEs and SAEs to resolution or stabilization in the clinical judgment of the study investigator.
13. Serum pregnancy test in all women. FSH only in post-menopausal women.
14. Females to use appropriate contraception and avoid pregnancy to 30 days after last study visit. Males to use appropriate contraception and refrain from donating sperm for 90 days after last study visit.

APPENDIX B: ACCEPTABLE RANGES OF SCREENING LABORATORY AND VITAL SIGN MEASUREMENTS

HEMATOLOGY AND CHEMISTRY

Lab test name	Reference range	Acceptable lower limit	Acceptable upper limit	Lab unit
Hemoglobin (Hgb), male	14-18	13.5	18.5	g/dL
Hemoglobin (Hgb), female	12-15	11.5	16.5	g/dL
Hematocrit (Hct), male	42-52	40	54	%
Hematocrit (Hct), female	37-47	35	49	%
White blood cell count (WBC)	4-11	3.5	11.5	$x10^3/\mu L$
Neutrophil count	1.4-8.2	1.2	8.5	$x10^3/\mu L$
Lymphocyte count	1-4.8	0.8	5	$x10^3/\mu L$
Monocyte count	0-0.9	0	1.1	$x10^3/\mu L$
Eosinophil count	0-0.3	0	0.6	$x10^3/\mu L$
Basophil count	0-0.2	0	0.3	$x10^3/\mu L$
Platelet count	150-400	125	475	$10^9/L$
Sodium	137-145	132	148	mmol/L
Potassium	3.5-5.1	3.3	5.3	mmol/L
Bicarbonate (CO_2)	22-30	20	32	mmol/L
Chloride	98-107	94	115	mmol/L
Calcium	8.4-10.2	8.0	11.0	mg/dL
Magnesium	1.6-2.3	1.1	2.8	mg/dL
Blood urea nitrogen (BUN)	7-20	0	25	mg/dL
Phosphorous	2.5-4.5	2.3	4.8	mg/dL
Glucose, fasting	74-106	50	120	mg/dL
Serum creatinine	0.5-1.3	<0.5	1.4	mg/dL
Direct Bilirubin	0.0-0.4	0	0.5	mg/dL
Total bilirubin	0.2-1.3	0	1.5	mg/dL
Total protein	6.3-8.2	6.0	8.6	g/dL
Albumin	3.5-5.0	3.2	6.0	g/dL
Aspartate transferase (AST)	3-42	0	46	U/L
Alanine transferase (ALT), male	21-72	0	80	U/L
Alanine transferase (ALT), female	10-44	0	50	U/L
Alkaline phosphatase	38-126	0	150	U/L
Hepatitis B surface antigen	non-reactive	non-reactive	non-reactive	n/a
Hepatitis C antibodies	non-reactive	non-reactive	non-reactive	n/a
HIV test	non-reactive	non-reactive	non-reactive	n/a
Serum β-HCG (females only)	negative	negative	negative	n/a
FSH (post-menopausal)	21.5-131	21.5	131	mIU/mL

Footnotes: Exceptions to screening laboratory tests' normal reference ranges are:

a. Racially-based low total WBC or neutrophil counts up to toxicity Grade 1 in Appendix C Toxicity Tables, are allowed, but toxicity Grades 2 or 3 are exclusionary.
b. Abnormalities in mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), mean platelet volume (MPV), and nucleated red blood cell count (NRBC CT), which are included in a CBC with differential, will not be exclusionary. The results will be included in the database for comparison with results obtained as a part of safety laboratory tests.
c. Abnormalities in serum creatinine, BUN, total bilirubin, AST, ALT, and chloride below the lower limit of normal (LLN) are allowable.
d. Elevated bilirubin due to documented Gilbert's syndrome that is Grade 1 is allowable, but Grade 2 or higher is exclusionary. (To document a subject has Gilbert syndrome, a diagnosis from the medical record must be provided or PI may make a 'presumptive' diagnosis of Gilbert syndrome in subjects with unconjugated hyperbilirubinemia on repeated testing (at least two samples separated in time) who have otherwise normal serum ALT, AST, and AP concentrations, and a normal CBC.
e. Elevated serum glucose, sodium, potassium, bicarbonate (CO_2), total protein, and AP, with toxicity Grade 1 values, are allowable.
f. Serum chloride and albumin above the upper limit of normal (ULN) is allowable.
g. Other laboratory values that are outside the range of eligibility but are thought to be due to an acute condition or due to collection or laboratory error may be repeated once.

URINALYSIS [a]

Lab test name	Reference range limit	Acceptable lower limit	Acceptable upper limit	Lab unit
Urine dipstick				
Protein	negative	negative	1+ [b]	
Blood	negative	negative	trace [b]	
Glucose	negative	negative	trace [b]	
Urine Microscopy				
WBCs in urine (sediment)	0-5	0	6	/field
RBCs in urine (sediment)	0-5	0	6	/field
Bacteria	negative	few	[b]	/field
Hyaline casts	0.5	none	trace [b]	/field
Crystals	none	0	[b]	

Footnotes:

[a] *(1) If UA by dipstick is abnormal, a complete UA with microscopic evaluation will be performed and the results will supersede the results of the dipstick.*
(2) Menstruating females failing inclusion criteria due to a positive blood on a urine test (dipstick or microscopic UA) may be retested following cessation of menses. Do not exclude female subjects with ≤ 6 RBC/HPF.

[b] Per investigator judgment, based on medical history.

TOXICOLOGY

Urine must be negative for all tested substances (amphetamines, cocaine (and metabolite), barbiturates, benzodiazepines, opiates, tetrahydrocannabinol, methamphetamines, methadone, TCA, MDMA, and phencyclidine). Urine creatinine will be measured as part of the profile to assess quality of collected sample.

VITAL SIGNS

Measurement	lower limit	upper limit	lab unit
Systolic BP	90	150	mmHg
Diastolic BP	40	90	mmHg
Pulse rate	45	100	beats/min
Respiratory rate	8	22	breaths/min
Oral temperature	95.9 (35.5)	<100.4 (<38.0)	°F (°C)

APPENDIX C: ADVERSE EVENTS TOXICITY GRADING CRITERIA

ABBREVIATIONS: Abbreviations utilized in the Table:

LLN = Lower Limit of Normal
Req = Required
IV = Intravenous
Dec = Decreased

ESTIMATING SEVERITY GRADE

For abnormalities NOT found elsewhere in the Toxicity Tables, use the scale below to estimate grade of severity:

GRADE 1	**Mild**	Events require minimal or no treatment; do not interfere with the subject's daily activities.
GRADE 2	**Moderate**	Events result in a low level of inconvenience or concern with therapeutic measures; may cause some interference with functioning.
GRADE 3	**Severe**	Events interrupt a subject's usual daily activity and may require systemic drug therapy or other treatment; are usually incapacitating.

SERIOUS OR LIFE-THREATENING AEs

Clinical events considered to be serious or life-threatening include, but are not limited to: seizures, coma, tetany, diabetic ketoacidosis, disseminated intravascular coagulation, diffuse petechiae, paralysis, acute psychosis, and severe depression.

COMMENTS REGARDING THE USE OF THESE TABLES

- Standardized and commonly used toxicity tables (Division of AIDS, National Cancer Institute [NCI] Common Toxicity Criteria [CTC], and World Health Organization [WHO]) have been adapted for use by the Division of Microbiology and Infectious Diseases (DMID) and modified to better meet the needs of subjects in DMID trials.
- For parameters not included in the following Toxicity Tables, the site should refer to the "Guide for Estimating Severity Grade" located above.
- Criteria are generally grouped by body system.

Some protocols may have additional protocol specific grading criteria, which will supersede the use of these tables for specified criteria.

Toxicity Grading Tables

Clinical AEs	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Comments
VITAL SIGNS				
Fever - °C Fever - °F	38.0-38.4 100.4-101.1	38.5-38.9 101.2-102.0	>38.9 >102.0	No recent hot or cold beverages or smoking. A protocol should select either ^{0}C or ^{0}F for inclusion
Tachycardia - bpm	101-115	116-130	>130 or ventricular dysrhythmias	Assume awake and in supine position for 5 min at rest; for AE, measurements at least 3 times with 2 concordant results (Section 8.1.6)
Bradycardia - bpm	50-54 OR 45-50 if baseline <60	45-49 OR 40-44 if baseline <60	<45 OR <40 if baseline <60	as above
Hypertension (systolic) - mm Hg	141-150	151-160	>160	Assume awake, and in supine position for 5 min at rest; for AE, measurements on same arm at least 3 times with 2 concordant results (Section 8.1.6)
Hypertension (diastolic) - mm Hg	91-95	96-100	>100	As above
Hypotension (systolic) - mm Hg	85-89	80-84	<80	As above
Tachypnea – breaths per min	23-25	26-30	>30	Assume awake and in supine position for 5 min at rest; for AE, measurements at least 3 times with 2 concordant results (Section 8.1.6)

Note: Isolated/individual abnormalities of vital signs would not be considered toward halting criteria. Abnormalities of vital signs should be described as "increase X" or "decrease X" (X = heart rate, blood pressure, respiratory rate, temperature) if asymptomatic, transient and not associated with a systemic or organ-specific disorder, and coded by MedDRA within the System Organ Class (SOC) "Investigations." These abnormalities should be graded per criteria in Appendix C, but not considered in determining whether study stopping criteria have been met. On the other hand, abnormalities of vital signs that are either

secondary to systemic or organ-specific clinical syndrome or primary disorders should be coded in the appropriate SOC (e.g., "cardiac disorders", respiratory disorders", "immunological disorders", etc.). These abnormalities should be considered in determining whether stopping criteria have been met.

Clinical AEs	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Comments
CARDIOVASCULAR				
Arrhythmia		Asymptomatic or transient signs; no medical intervention required	Recurrent and/or persistent signs; symptomatic medical intervention required	
Hemorrhage	Estimated blood loss ≤100 mL	Estimated blood loss >100 mL; no transfusion required	Blood transfusion required	
RESPIRATORY				
Cough	Transient cough; no treatment required	Persistent cough; treatment required	Interferes with daily activities	
Bronchospasm, Acute	Transient bronchospasm; no treatment required; FEV1 71-80% of predicted peak flow	Requires treatment; normalizes with bronchodilator; FEV1 60-70% of predicted peak flow	No normalization with bronchodilator; FEV1 <60% of predicted peak flow	
Dyspnea	Does not interfere with usual and social activities	Interferes with usual and social activities; no treatment	Prevents usual and social activities, OR requires treatment	
GASTROINTESTINAL				
Nausea	No interference with normal activity	Some interference with normal activity	Prevents daily activities	
Vomiting	No interference with activity, OR 1-2 episodes in a 24-h period	Some interference with activity, OR >2 episodes in a 24-h period	Prevents daily activity, OR requires medical intervention	

Clinical AEs	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Comments
Diarrhea	2-3 loose or watery stools in a 24-h period	4-5 loose OR watery stools in a 24-h period	6 or more loose or watery stools in a 24-h period, OR requires IV hydration OR requires medical intervention	
Oral Discomfort / Dysphagia	Mild discomfort; no difficulty swallowing	Some limits on eating / drinking	Eating / talking very limited; unable to swallow solid foods	
LOCAL IV CATHETER REACTION				
IV site reaction	Not Applicable	Erythema with associated symptoms (e.g., edema, pain, induration, phlebitis)	Ulceration or necrosis; severe tissue damage; operative intervention indicated	
SYSTEMIC REACTIONS				
Anaphylaxis **	--	--	Symptomatic bronchospasm, with or without urticaria; parenteral intervention indicated; allergy-related edema or angioedema; hypotension	
***Definition*: A disorder characterized by an acute inflammatory reaction resulting from the release of histamine and histamine-like substances from mast cells, causing a hypersensitivity immune response. Clinically, it presents with breathing difficulty, dizziness, hypotension, cyanosis and loss of consciousness and may lead to death.*				
Allergic Reaction	Pruritus without rash	Localized urticaria OR requires oral therapy	Generalized urticaria; angioedema OR anaphylaxis OR requires epinephrine	

Clinical AEs	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Comments
Hypersensitivity (including drug fever)	Transient flushing or rash; temperature 38.0-38.4 °C (100.4-101.1°F)	Rash; flushing; urticaria; dyspnea; temperature 38.5 - 38.9°C (101.2 – 102.0°F)	Symptomatic bronchospasm with or without urticaria; parenteral medication indicated; allergy-related edema or angioedema; hypotension; temperature >38.9°C (>102.0°F)	
Headache	No interference with activity	Repeated use of non-narcotic pain reliever for more than 24 h OR some interference with activity	Significant; any use of narcotic pain reliever OR prevents daily activity OR requires triptans	
Fatigue	No interference with activity	Some interference with activity	Significant; prevents daily activity	
Myalgia	No interference with activity	Some interference with activity	Significant; prevents daily activity	
SKIN				
Mucocutaneous	Erythema, pruritus	Diffuse, maculo-papular rash, dry desquamation	Vesiculation OR moist desquamation OR ulceration	
Pruritus	No or minimal interference with usual social and functional activities	Greater than minimal interference with usual social and functional activities	Inability to perform usual social and functional daily activities	
ALL OTHER CONDITIONS				
Illness or clinical AE (as defined according to applicable regulations)	No interference with activity	Some interference with activity but not requiring medical intervention	Prevents daily activity and requires medical intervention	

Laboratory AEs	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Blood, serum, or plasma *			
Sodium decrease – mmol/L	130 –132	125 –130	<125
Sodium increase – mmol/L	148 – 149	150 – 153	≥154
Potassium increase – mmol/L	5.3 – 6.0	6.1 – 6.4	≥6.5
Potassium decrease – mmol/L	3.0 – 3.3	2.5 – 2.9	<2.5
Bicarbonate (CO_2) increase – mmol/L	32 - 34	35 – 36	>36
Bicarbonate (CO_2) decrease – mmol/L	18 -- 20	14 - 17	<14
Glucose decrease, fasting – mg/dL	50 – 73	45 – 49	<45
Glucose increase, fasting – mg/dL	107– 125	126 - 249	≥250
Glucose increase, non-fasting – mg/dL	107- 160	161 - 249	≥250
Blood urea nitrogen – mg/dL	21 – 26	27 – 31	>31
Creatinine increase – mg/dL	1.4 – 1.7	1.8 – 2.3	>2.3
Calcium decrease – mg/dL	7.8 –8.0	7.0 – 7.7	<7.0
Calcium increase – mg/dL	11.0 – 11.4	11.5 – 12.4	≥12.5
Phosphorous increase – mg/dL	4.8 – 5.0	5.1 – 5.5	>5.5
Phosphorous decrease – mg/dL	2.0 – 2.4	1.4 – 1.9	<1.4
Total protein decrease – g/dL	5.2 – 6.0	4.8 – 5.1	<4.8
Albumin decrease – g/dL	2.8 – 3.4	2.5 – 2.7	<2.5
AST increase – U/L	43 – 104	105 – 209	≥210
ALT increase, male – U/L	73 – 179	180 – 359	≥360
ALT increase, female – U/L	45 – 109	110 – 219	≥220
Alkaline phosphatase increase – U/L	151 – 240	241 – 360	>360
Total bilirubin (serum) increase – mg/dL (with other LFTs in the normal range)	1.4 – 2.0	2.1 – 2.5	>2.5
Total bilirubin (serum) increase – mg/dL (accompanied by a >3 x ULN increase in ALT or AST)**	1.4 – 1.6	1.7 – 2.0	>2.0
Hemoglobin decrease, female – g/dL	11.0 – 11.5	9.5 – 10.9	< 9.5
Hemoglobin decrease, male – g/dL	12.0 –13.5	10.0 – 11.9	<10.0
WBC increase – cell/mm^3	11,000 – 15,000	15,001 – 20,000	>20,000
WBC decrease – cell/mm^3	2,500 –3,500	1,500 – 2,499	<1,500
Neutrophils decrease – cell/mm^3	1,200 – 1,399	1,000 – 1,199	<1,000
Lymphocytes decrease – cell/mm^3	750 – 999	500 – 749	<500
Monocytes increase – cell/mm^3	1,101 – 2,000	2,001– 3000	>3,000
Eosinophils increase – cell/mm^3	500 – 750	751 – 1,500	>1,500
Basophils increase – cell/mm^3	201 – 500	501 – 800	>800
Platelets decrease – cell/mm^3	120,000 – 125,000	100,000 – <120,000	<100,000

Laboratory AEs	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Urine*			
Protein	1+	2+	>2+
Glucose	1+	2+	>2+
Blood (dipstick)	1+	2+	>2+
Blood (microscopic) - RBCs per HPF	6-10	11-50	>50 and/or gross blood
WBC (microscopic) – WBC per HPF	5-10	11-50	>50
Bacteria (microscopic)	few	moderate	many

* Institutional normal reference ranges and allowable ranges at screening are provided in Appendix B.
**ALT, males: >216 U/mL; ALT, females: >132 U/mL; AST, male or female: >126 U/mL)
Note 1: If a subject was accepted into the trial with a laboratory value of an analyte that overlaps with values used for grading Grade 1 (mild) laboratory abnormalities, an AE will be reported if the on-study value of the same analyte is different (worse) from the baseline.
Note 2: Safety laboratory results that are abnormal according to the local laboratory reference range, but not considered a Grade 1 abnormality, will be evaluated by the study site clinician and reported as Grade 1 abnormality if clinically significant. If not clinically significant, these will not be considered laboratory AEs and will thus not be graded, but will be recorded in the source document and followed-up clinically at the discretion of the study site clinician.
Note 3: Other laboratory parameters performed and reported as parted of the complete blood count, metabolic panel and urinalysis will be evaluated by the study physician, recorded in the source document, and reported as laboratory AEs if clinically significant, and graded according to the criteria in Section 9.2.1.
Note 4: If US by dipstick is abnormal, a microscopic UA will be performed and the results will supersede the results of the dipstick UA.
Note 5: Menstruating females with a positive urine dipstick or microscopic UA may be retested following cessation of menses.

ECG interval abnormality	Grade 1	Grade 2	Grade 3
QT/QTc interval (Fridericia's correction) prolonged (msec)	Asymptomatic, QTc 450-479 msec, OR increase in interval <30 msec above baseline	Asymptomatic, QTc 480-499 msec OR increase in interval 30-59 msec above baseline	Asymptomatic, QTc ≥500 msec OR increase in interval ≥60 msec above baseline
PR interval prolonged - sec	0.21-0.25 sec	>0.25 sec	Type II 2nd degree AV block OR ventricular pause >3.0 sec

Note: The events will be coded as SAE if there are life-threatening associated symptoms or signs (arrhythmia, CHF, hypotension, syncope, torsade's de pointes, etc.)
Note: If a male subject was accepted into the trial with a QT/QTc value that overlaps with values used for grading Grade 1 (mild) QT/QTc prolongation, an AE will be reported if the on-study value of the QT/QTc is higher than the baseline.

APPENDIX D: BLOOD VOLUME WITHDRAWN DURING THE TRIAL

Laboratory Samples and Estimated Total Blood Volume (mL)

Study visit	SCREENING	CHECK-IN	INPATIENT PERIOD				FINAL	Total volume	Early term visit
Study Day	-21 to -2	-1	1	2	3	4	8 (±2)		
HEMATOLOGY [1]	4	4				4		12	4
CHEMISTRY and serum β-HCG, FSH, HIV, HBsAg, HCV [1]	5	5				5		15	5
PK [2]			54	12	6	6		78	6
Total volume/visit	9	9	54	12	6	15	0	-	15
Cumulative total volume	9	18	72	84	90	105	105	105	

[1] Clinical blood tests are drawn at Screening Visit, on Day-1, and on Day 4, or ET.
[2] PK serum samples are drawn on Day 1: 30 min before dosing and at 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 36, 48 and 72 h after dosing, or ET.