A Phase 1, Randomized, Double-blinded, Four-period Crossover, Thorough QT/QTc (TQT) Clinical Trial to Evaluate the Effect of Zoliflodacin on Cardiac Repolarization in Healthy Male and Female Subjects

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Statement of Compliance

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

- United States (US) 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 Council for Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use E6(R2) Good Clinical Practice (ICH E6 GCP): Integrated Addendum to ICH E6(R1) Guidance for Industry, published in the Federal Register (83 Federal Register 8882 (2018)), including the latest finalized.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 subjects are protected, consistent with principles that originate 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 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
β-HCG	Beta Human Chorionic Gonadotropin
BID	Twice-Daily
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
CHEM	Chemistry Panel
CI	Confidence Interval
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
DCC	Data Coordinating Center
ΔΔQTcF	Time-matched, placebo-corrected, baseline-adjusted difference in QTcF interval
dL	Deciliter
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
GFR	Glomerular Filtration Rate
h	Hour(s)
HBsAg	Hepatitis B Surface Antigen
Hct	Hematocrit
HCV	Hepatitis C Virus
HEENT	Head, Eyes, Ears, Nose, and Throat
HEM	Hematology Tests
Hgb	Hemoglobin
HIV	Human Immunodeficiency Virus
HPF	High-Powered Field
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 Council for 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(s)
LLN	Lower Limit of Normal
LQTS	Long QT Syndrome
MDMA	3,4-methylenedioxy-methamphetamine
MedDRA®	Medical Dictionary for Regulatory Activities
µg	Microgram(s)
mg	Milligram(s)
MH	Medical History
MIC	Minimum Inhibitory Concentration
min	Minute(s)
mL	Milliliter(s)
MM	Medical Monitor
mmHg	Millimeters of Mercury
µmol	Micromole(s)
MOP	Manual of Procedures
msec	Milliseconds(s)
mV	Millivolt
N	Number (typically refers to the total number of subjects)
n	Number (typically refers to a subset of the total number of subjects)
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
NOAEL	No Observable Adverse Effect Level
NSAID	Nonsteroidal Anti-Inflammatory Drug
OCRR	Office of Clinical Research Resources
OTC	Over-the-Counter
PBPK	Physiologically-Based Pharmacokinetic
PD	Pharmacodynamic(s)
PE	Physical Examination
PI	Principal Investigator
PK	Pharmacokinetic(s)
PO	Oral

POC	Point of Contact
PVG	Pharmacovigilance Group
QTc	Corrected QT Interval of the ECG
QTcF	Corrected QT interval of the ECG using Fridericia's Formula
RBC	Red Blood Cell
RP	Research Pharmacist
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SD	Standard Deviation
sec	Second(s)
SMC	Safety Monitoring Committee
TCA	Tricyclic Antidepressant
TdP	Torsade de Pointes
T_{max}	Time to Maximum Plasma Concentration
TQT	Thorough QT/QTc
UA	Urinalysis
ULN	Upper Limit of Normal
US	United States
VS	Vital Signs
WBC	White Blood Cell
WHO	World Health Organization

Protocol Summary

Title:	A Phase 1, Randomized, Double-blinded, Four-period Crossover, Thorough QT/QTc (TQT) Clinical Trial to Evaluate the Effect of Zoliflodacin on Cardiac Repolarization in Healthy Male and Female Subjects
Phase:	1
Population:	72 randomized (at least 56 evaluable) healthy male and female subjects aged 18 to 45 years inclusive
Number of Sites:	One (Vince & Associates Clinical Research, Inc., Overland Park, KS)
Study Duration:	Approximately 12 weeks
Subject Participation Duration:	Up to 55 days (from Screening Visit to Final Visit) The trial will consist of: • A Screening Period of up to 20 days (Day -21 to Day -2); • Four Dosing Periods, each lasting 3 days/2 nights (Days -1, 1, and 2 in each period), totaling 12 days; • Four Outpatient Periods, each lasting 5 days (Days 3-7 after each Dosing Period), totaling 20 days; and • A Final Visit 8 ±2 days after dosing in Dosing Period 4, totaling 1 day.
Description of Agents:	The following four treatments will be administered to all subjects using a randomized, double-blinded, four-period, crossover design. Each subject will be randomized to a treatment sequence containing all four treatments: • Treatment A: zoliflodacin 2 g (investigational product) • Treatment B: zoliflodacin 4 g (investigational product) • Treatment C: placebo (for zoliflodacin) • Treatment D: moxifloxacin 400 mg (positive comparator) Single 2-g and single 4-g doses of zoliflodacin were safe and well-tolerated in previous clinical trials. The 2-g dose (of the new formulation) has been selected as the therapeutic dose for further clinical testing of zoliflodacin. The 4-g dose was shown to result in drug exposures higher than the 2-g dose and should provide exposures that will be achieved in patients when used with other drugs that may interact with zoliflodacin. Based on pharmacokinetic

(PK) data from study DMID 16-0118, exposures for 4 g of the new formulation are similar to those for the previous formulation. For this reason, the 4-g dose was selected as the supratherapeutic dose for this TQT trial. Placebo for zoliflodacin was selected to control for natural diurnal changes in the electrocardiogram (ECG). Moxifloxacin was selected as a positive comparator to evaluate QTcF assay sensitivity, as it predictably causes a mild prolongation of the QTcF interval but has a very low risk for inducing cardiac arrhythmias.

Objectives:

Primary:

- To evaluate the effect of zoliflodacin on the corrected QT interval of the ECG using Fridericia's formula (QTcF)

Secondary:

- To evaluate the effects of zoliflodacin on other ECG parameters [PR, QRS, and RR intervals, and heart rate (HR)]
- To evaluate the sensitivity of QTcF measurement using moxifloxacin
- To evaluate the effect of zoliflodacin on T-wave morphology
- To evaluate the PK of 2 g and 4 g oral zoliflodacin under fasting state
- To evaluate the relationship between zoliflodacin PK and time-matched QTcF pharmacodynamics (PD)
- To evaluate the safety and tolerability of 2 g and 4 g oral zoliflodacin

Outcome Measures:

Primary:

- The upper bound of the one-sided 95% confidence interval (CI) for the largest time-matched, placebo-corrected, baseline-adjusted mean QTcF interval (ΔΔQTcF) collected in the 24-h period after 2 g and 4 g dose of zoliflodacin is <10 msec

Secondary:

- Time-matched, placebo-corrected, baseline-adjusted non-QT intervals (PR, QRS, and RR intervals) and HR collected in the 24-h period after 2 g and 4 g dose of zoliflodacin
- The lower bound of the one-sided 95% CI of the ΔΔQTcF is >5 msec at one or more individual time points between 1 h and 4 h after a single dose of moxifloxacin 400 mg
- Incidence of abnormal T-wave morphology using defined categories in the 24-h period after 2 g and 4 g dose of zoliflodacin

- Single-dose plasma concentrations (and PK exposure parameters, such as C_{max} and AUC) of zoliflodacin collected up to 24 h after 2 g and 4 g dose of zoliflodacin
- Relationship between plasma concentrations of zoliflodacin (and PK exposure parameters, such as C_{max} and AUC) and ΔΔQTcF up to 24 h after 2 g and 4 g dose of zoliflodacin
- Occurrence of treatment-emergent serious adverse events (SAEs), other unsolicited treatment-emergent adverse events (AEs), and changes from baseline in vital signs (VS), clinical laboratory values, and ECG parameters following administration of zoliflodacin and moxifloxacin from time of dosing to Final Visit, or Early Termination (ET) Visit.

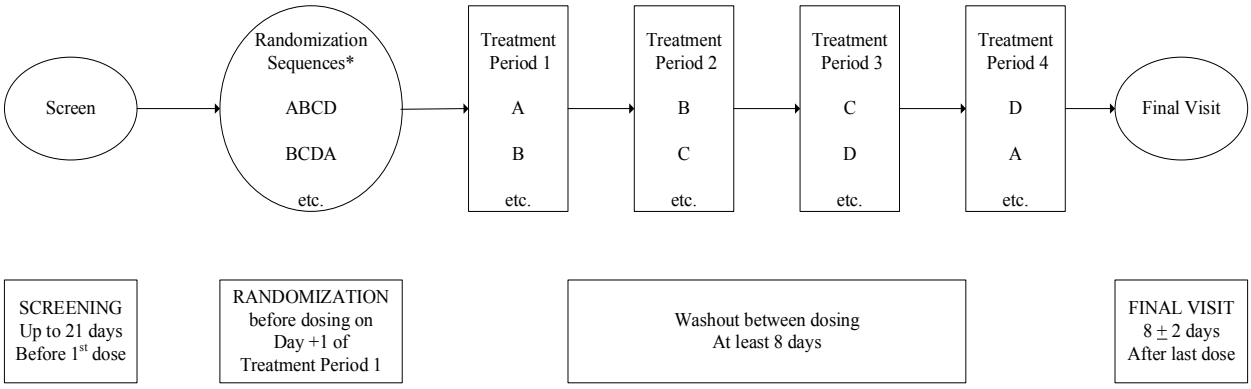
Description of Study Design:

The TQT clinical trial will be performed according to a randomized, double-blinded (except for the use of moxifloxacin), placebo-controlled, four-period, four-treatment, crossover design in 72 healthy male and/or female subjects.

Subjects who consent to participate will be enrolled in the trial if they meet all inclusion and no exclusion eligibility criteria. Laboratory assessments at Screening Visit will include clinical laboratory tests [hematology (HEM), chemistry (CHEM), and urinalysis (UA)], testing for viral serology [human immunodeficiency virus (HIV) antibody, hepatitis B surface antigen (HBsAg), and hepatitis C virus (HCV) antibody], drugs of abuse, and alcohol. Serum pregnancy testing will be done in all females and serum follicle-stimulating hormone (FSH) levels will be measured only in post-menopausal females. Subjects who meet eligibility criteria will be randomly allocated to one of 12 dosing sequences and receive a single dose of the four study drugs according to their randomly-assigned dosing sequence during four dosing periods. The dosing interval between dosing periods will be 8 days to allow for adequate wash-out of drug before the next dose. Safety assessments at scheduled visits after dosing will include medical history (MH), VS, physical examination (PE), 12-lead ECG, and clinical laboratory tests. Continuous 12-lead Holter ECGs will be initiated 1 h before dosing and continue for 24 h after dosing. At scheduled timepoints, replicate Holter ECGs will be selected for measurement of QTcF and other ECG intervals, and assessment of T-wave morphologies. Plasma will be collected during each dosing period at scheduled timepoints that are time-matched to Holter ECGs before and after dosing on Day 1 and through Day 2 (24 h after dosing), or ET if within 24 h after dosing, but drug concentration bioanalysis and PK analysis will be done only for zoliflodacin. Treatment-emergent AEs and SAEs will be assessed from time of dosing to end of trial.

Estimated Time to Complete Enrollment:	Up to 12 weeks (from dosing of first subject to Final Visit of last subject)
Halting Criteria Decision Process:	If criteria for halting the trial (as listed in Section 9.5.1) are met following dosing in any dosing period, administration of all study drugs will be suspended and 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 safety assessments in future clinical trials with zoliflodacin.

Figure 1: Description Schematic of Study Design



NOTE: A, B, C, and D refer to each of the study treatments. This is a four-treatment, four-period, crossover trial. Treatments and treatment sequences will be randomized using a 12-sequence design balanced for period and first-order carryover effects, and double-blinded, except for administration of moxifloxacin. Six subjects will be randomized to each treatment sequence. The schematic displays only two of the 12 sequences.
* See Section 4 for details and a complete list of planned treatment sequences.

1 KEY ROLES

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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 (previously called 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. Detailed nonclinical and clinical data are provided 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 [redacted] This novel mode of inhibition is further supported by the observation that [redacted] *N. gonorrhoeae* resistance to zoliflodacin has been mapped 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 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 area under the curve (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 Phase 2, multi-center, randomized trial to assess the safety and efficacy of oral zoliflodacin to treat uncomplicated urogenital gonorrhea in adults compared to intramuscular (IM) ceftriaxone. Subjects were randomly assigned to receive a single oral dose of 2 g of zoliflodacin, 3 g of zoliflodacin, or a single IM dose of 500 mg of ceftriaxone. In the per-protocol population, microbiological cure was observed in 96% and 100% in patients treated with 2 g and 3 g of zoliflodacin, respectively.[8]

Human Pharmacokinetics

A Phase 1, single-center, multi-part trial with an oral suspension of zoliflodacin vs. 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 time to maximum plasma concentration (T_{max}) ranging from [redacted] Following maximum plasma concentration (C_{max}), plasma drug concentrations [redacted] The terminal elimination phase started at approximately [redacted] zoliflodacin concentrations [redacted] Exposures [redacted] suggesting a [redacted] When [redacted] zoliflodacin was administered, [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, 2-g and 3-g doses of zoliflodacin were selected to maximize the probability of achieving the target plasma [redacted] The AUC and C_{max} values for zoliflodacin at the various doses also [redacted] values.[8]

The PK of a single 4-g dose of zoliflodacin, packaged in the new formulation that will be used in the current trial, was evaluated in a recent Phase 1 trial in healthy subjects (DMID 16-0118) and was compared to PK data obtained in the previously completed trials.[8,12] This analysis demonstrated that the PK of the 4-g dose of the new formulation of zoliflodacin is similar to the previous one, and higher exposure at the 4-g dose compared to the 2-g dose.

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 extensively metabolized via cytochrome P450- and non-P450-mediated pathways. [redacted] The potential for drug-drug interactions has been evaluated using a physiologically-based PK (PBPK) model developed with [redacted].[8] In summary:

1. Zoliflodacin as a victim: Single doses of zoliflodacin ranging from [redacted] are predicted to [redacted] based upon Phase 1 PK data in healthy subjects, [redacted]

[redacted] Since the primary PK driver for efficacy of zoliflodacin is [redacted].

2. Zoliflodacin as a perpetrator: Zoliflodacin is predicted to increase [redacted]. The extent of the interaction is not predicted to differ between the zoliflodacin [redacted]. This interaction [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 torsade de pointes (TdP), 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 – [redacted] There was [redacted] on any evaluated endpoints including [redacted] were observed up to the highest dose tested. [redacted] were evident at either primary or recovery necropsy. [redacted] was established at [redacted] and correlated with [redacted] At this highest dose tested, [redacted] was observed, consistent with [redacted] These effects were [redacted]

can be monitored in the clinical setting. There were [redacted] with zoliflodacin administration. [redacted], there were [redacted].[8]

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

[redacted] were given zoliflodacin at [redacted] there was [redacted] there was [redacted] was considered to be [redacted].

The effects of zoliflodacin on male fertility have been assessed in [redacted] were dosed [redacted]. Administration at [redacted] was associated with a [redacted] which was [redacted].

Human trials: Four trials have evaluated the safety of zoliflodacin in humans.

In healthy subjects in the Phase 1 trials, [redacted] The most common AEs in the zoliflodacin groups were [redacted] There were [redacted].[8,12]

In patients in the Phase 2 trial, zoliflodacin was generally safe and well-tolerated. Reported AEs 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 with zoliflodacin 3 g (9%) compared to zoliflodacin 2 g (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 3-g groups combined and excluding infection-related AEs) were diarrhea, headache, and nausea.[8,13]

In a recently completed Phase I open-label clinical trial (DMID 16-0118), a single 4-g dose of zoliflodacin formulated by the new method was shown to be safe and well-tolerated, and there were no differences in the safety profile from earlier studies.

2.2 Rationale

2.2.1 Study Rationale

The current clinical trial will be conducted to assess the effect of zoliflodacin on cardiac repolarization. Medicinal products that prolong cardiac repolarization may have a proarrhythmic effect and have been associated with a specific, potentially fatal polymorphic ventricular tachycardia termed torsade de pointes (TdP). This tachycardia is usually observed in the setting of a prolonged QT interval, is often initiated after extrasystolic pauses, and is identified by the continuously twisting appearance of the QRS complex in the 12-lead ECG recording. Additional risk factors associated with a potential predisposition to the proarrhythmic effect include cardiac disease, congenital Long QT Syndrome (LQTS), hypokalemia, hypomagnesemia, bradycardia, and concurrent administration of other medicinal products known to prolong the QT interval.

The QT interval, which represents ventricular recovery time, reflects the time between the depolarization of the first myocardial cells and the completion of repolarization of the last myocardial cells. Like ventricular refractoriness, it is influenced by the autonomic nervous system and changes in HR, and also by electrolyte disturbances and medications.[14,15] The duration of the QT interval has prognostic implications in both healthy and diseased populations, with an abnormally prolonged QT interval becoming an increasingly accepted marker of risk for malignant ventricular tachyarrhythmias.[16]

There are a number of compounds in various therapeutic classes that have proarrhythmic potential, including, among others, imidazoles, psychotropic agents, antihistamines, and antibiotics.[17] Of particular concern is concurrent administration of other medicinal products known to prolong the QT interval and/or that are metabolized by and compete for cytochrome P4503A4 (CYP3A4) enzymes. A partial list of drugs considered to be associated with high-risk of TdP when associated with QTc prolongation is presented in Appendix E: A Partial List of Drugs Causing QTc Prolongation and Torsade de Pointes.

Regulatory guidance has emphasized the need to obtain clear robust data on the effect of new chemical entities on ECG parameters with focus on cardiac repolarization as measured by the corrected QT interval (QTc) duration.[16] Though many Phase 1, 2, and 3 studies may be conducted, they usually have insufficient sample size, infrequent sampling of ECG data, or the use of inadequate controls to overcome the high rate of spontaneous change in QTc duration.

This has resulted in regulatory guidance recommending a dedicated TQT study to define the ECG effects of new drugs.[16]

Therefore, this four-arm crossover trial will include therapeutic and supratherapeutic dosing with zoliflodacin, placebo as a negative control, and oral moxifloxacin as a positive control. The trial will be conducted in accordance with relevant ICH E14 and Food and Drug Administration (FDA) guidance.[16]

The positive control (moxifloxacin) will serve to validate the sensitivity of the assay to detect small increases from baseline in the QTc interval corrected using Fridericia's formula (QTcF). Moxifloxacin will be used because it has been demonstrated to increase QTc by a mean of 6 msec (time-averaged analysis: standard deviation (SD) = 26 msec; N = 787) after a 400-mg oral dose.[16,18] However, thorough ECG studies have shown that this dose of moxifloxacin can be expected to show, for a time-matched analysis, a mean change of 10-15 msec, generally within 1-6 h after dosing.[19]

This trial will be performed in healthy subjects to eliminate variables known to change ECG parameters (e.g., concomitant drugs or diseases) among subjects. The sample size is driven by the need to do a time-matched statistical analysis on the primary endpoint as per ICH E14 guidance. A crossover study design was chosen so that subjects will act as their own controls. A crossover trial can be conducted since the half-life of zoliflodacin is approximately 6 h. A wash-out phase of 8 ±2 days after Day 1 of each dosing period is considered sufficient to prevent a carryover effect of the study drug between treatments.

2.2.2 Rationale for Dose Selection

The zoliflodacin 2-g dose chosen for this trial is intended to achieve the drug exposure achieved in gonorrhea patients for the anticipated therapeutic single dose of 2-3 g. Several elements were considered in the selection of the zoliflodacin supratherapeutic dose including the anticipated clinical usage, the Phase 1 data with the original formulation, including utilization of population PK modeling and PBPK modeling, the potential for drug-drug interactions to influence zoliflodacin C_{max}, and lack of availability of an IV formulation:

1. **Anticipated clinical usage**: Zoliflodacin is being studied as a single-dose oral therapy for the treatment of uncomplicated gonorrhea. The product is not intended for multiple-dose administration, which has not been studied. The C_{max} for zoliflodacin 4 g is anticipated to be supratherapeutic to peak concentrations observed for a therapeutic dose of 2-3 g when the drug is taken in the fasted state.
2. **Phase 1 data including utilization of population PK modeling and PBPK modeling**: In a Phase 1 study, in the dose range [redacted] The population PK model that has been developed adequately described the clinical data observed in healthy subjects [redacted]

[redacted][8] [redacted]. Based on the population PK model, therefore, [redacted]

3. **PBPK modeling**: In addition to the population PK model, a PBPK model incorporating physicochemical properties, absorption, distribution, and elimination of zoliflodacin was developed to investigate the systemic exposure of the drug after oral administration to healthy adults and determine the potential for drug-drug interactions [redacted][8]. The PBPK model was utilized to assess [redacted]. The model [redacted]. Based on these simulations, [redacted]
4. **Potential for drug-drug interactions to influence zoliflodacin C_{max} levels**: Using the PBPK model [redacted][8], the potential for zoliflodacin to be either a victim and/or a perpetrator in drug-drug interactions was evaluated. Although metabolic clearance of zoliflodacin is mediated predominantly by [redacted]. Based on these simulations, [redacted].
5. **The only formulation under development is the oral formulation**: There is no IV formulation available for use in the TQT study.

In the recently completed Phase 1 trial assessing PK parameters in healthy subjects [redacted]. Thus, the 4-g dose will be used as the supratherapeutic dose required by the ICH E14 Guidance for TQT studies.[16] Both the 2-g and 4-g doses of zoliflodacin were shown to be safe in previous studies. The 400-mg dose of moxifloxacin to be used is the highest dose recommended for use in the clinic and is commonly used as a positive control in TQT studies to ensure assay sensitivity for detecting potentially clinically significant changes in the QT/QTc interval.[16,20,21]

2.3 Identified and Potential Risks and Benefits

2.3.1 Zoliflodacin Risks

Identified Risks

There are no identified risks associated with zoliflodacin to date. 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.

Potential 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]
Continuous monitoring of [redacted] will be implemented in this trial.

In a Phase 1 and Phase 2 trial, no trends in [redacted]. Standard clinical monitoring [redacted].

[redacted]. The incidence of these findings typically [redacted] in the first-in-human trial. [redacted].

Effects of chronic dosing of zoliflodacin on [redacted] With respect to [redacted]

[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 before administration of zoliflodacin and at the end of the trial and will be required to use specified birth control methods during the trial and for 30 days after Final Visit.
- Male subjects will be required to use specified birth control methods during the trial and for 90 days after Final Visit and will be advised not to donate sperm during this period.

2.3.2 Moxifloxacin Risks

Moxifloxacin is a broad-spectrum 8-methoxy fluoroquinolone with activity against both Gram-positive and Gram-negative bacteria, including anaerobes. It is a licensed antibiotic for serious and life-threatening bacterial infections and can be used when other drugs fail. The maximum recommended dosage is 400 mg daily.[18, 22]

Overdose: Single oral overdoses up to 2.8 g were not associated with any SAEs. In case of acute overdose, the stomach should be emptied, and adequate hydration maintained. ECG monitoring is recommended due to the possibility of QT interval prolongation, and the patient should be carefully observed and given supportive treatment. The administration of activated charcoal as soon as possible after oral overdose may prevent excessive systemic moxifloxacin exposure.

Adverse reactions: AEs judged by investigators to be at least possibly drug-related, occurring in ≥2% of moxifloxacin-treated patients, were nausea (6%), diarrhea (5%), and dizziness (2%).

Electrocardiogram: Prolongation of the QT interval in the ECG has been observed in some patients receiving moxifloxacin. Following oral dosing with 400 mg of moxifloxacin the mean (± SD) change in QTc from the pre-dose value at the time of maximum drug concentration was 6 msec (± 26) (n = 787). Following a course of daily IV dosing (400 mg; 1-h infusion each day), the mean change in QTc from the Day 1 pre-dose value was 9 msec (± 24) on Day 1 (n = 69) and 3 msec (± 29) on Day 3 (n = 290). No cardiovascular morbidity or mortality attributable to QTc prolongation occurred with moxifloxacin treatment in over 9,200 patients in controlled clinical trials, including 223 patients who were hypokalemic at the start of treatment, and there was no increase in mortality in over 18,000 moxifloxacin tablet-treated patients in a post-marketing observational study in which ECGs were not performed.

In post-marketing studies, rare AEs were reported in patients treated with moxifloxacin. These include the following:

Tendinopathy and tendon rupture: Fluoroquinolones, including moxifloxacin, are associated with an increased risk of tendinitis and tendon rupture in all ages. This AE most frequently

involves the Achilles tendon, and rupture of the Achilles tendon may require surgical repair. Tendon rupture can occur during or after completion of therapy; cases occurring up to several months after completion of therapy have been reported.

Photosensitivity: Photosensitivity, which can appear as skin eruption or severe sunburn, can occur in some patients taking quinolone antibiotics after exposure to sunlight or artificial ultraviolet light (e.g., tanning beds).

Central nervous system: Quinolones may cause central nervous system events including convulsions, dizziness, confusion, tremors, hallucinations, depression, and, rarely, suicidal thoughts or acts. Nervousness, agitation, insomnia, anxiety, nightmares or paranoia have also been reported. These reactions may occur following the first dose.

Subjects will be closely monitored for all of these risks.

2.3.3 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.

2.3.4 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 17.5 mL at Screening Visit, 9 mL on Day -1, 69 mL during each inpatient period (Days 1-2), 9 mL at Final Visit, and 338.5 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, AE evaluation, 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 treatments (e.g., fluid replacement, good nutrition, vitamins, or iron supplementation).

2.3.5 Known Potential Benefits

The trial has no direct benefit for 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.

In accordance with the Health Insurance Portability and Accountability Act (HIPAA) regulation, subjects will be promptly notified of any clinical test results that would have an impact on their health. Screening evaluations may detect previously unknown abnormalities that could be clinically significant. This could benefit a subject, as it may lead to earlier diagnostic evaluation and treatment of an underlying disorder.

2.3.6 Risk/Benefit Ratio

This ratio cannot be calculated by available data. The risk for an SAE, including a significant ventricular arrhythmia, occurring in a subject treated with single doses of 2 g and 4 g of zoliflodacin or 400 mg of moxifloxacin is thought to be low. Subjects will be treated in an inpatient unit and closely monitored during the trial, so that emergency care can be provided immediately if an acute arrhythmia or other event occurs. In addition, should a significant QTcF prolongation occur, with or without TdP, the subject will receive appropriate treatment if needed, and counseled to obtain further consultation to rule out a genetic predisposition towards development of LQTS after using licensed drugs. Data collected in the clinical development of zoliflodacin to date, especially preliminary data in Phase 2 studies in patients with uncomplicated gonorrhea, support further development of zoliflodacin. The benefit of a potential new addition to the drugs available for the treatment of gonorrhea outweighs the known risks of the medications to be used in this trial.

3 OBJECTIVES AND OUTCOME MEASURES

3.1 Study Objectives

3.1.1 Primary

- To evaluate the effect of zoliflodacin on the corrected QT interval of the ECG using Fridericia's formula (QTcF)

3.1.2 Secondary

- To evaluate the effects of zoliflodacin on other ECG parameters (PR, QRS, and RR intervals, and HR)
- To evaluate the sensitivity of QTcF measurement using moxifloxacin
- To evaluate the effect of zoliflodacin on T-wave morphology
- To evaluate the PK of 2 g and 4 g oral zoliflodacin under fasting state
- To evaluate the relationship between zoliflodacin PK and time-matched QTcF PD
- To evaluate the safety and tolerability of 2 g and 4 g oral zoliflodacin

3.2 Study Outcome Measures

3.2.1 Primary

- The upper bound of the one-sided 95% confidence interval (CI) for the largest time-matched, placebo-corrected, baseline-adjusted mean QTcF interval (ΔΔQTcF) collected in the 24-h period after 2 g and 4 g dose of zoliflodacin is <10 msec

3.2.2 Secondary

- Time-matched, placebo-corrected, baseline-adjusted non-QT intervals (PR, QRS and RR intervals) and HR collected in the 24-h period after 2 g and 4 g dose of zoliflodacin
- The lower bound of the one-sided 95% CI of the ΔΔQTcF is >5 msec at one or more individual timepoints between 1 h and 4 h after a single dose of moxifloxacin 400 mg
- Incidence of abnormal T-wave morphology using defined categories in the 24-h period after 2 g and 4 g dose of zoliflodacin
- Single-dose plasma concentrations (and PK exposure parameters, such as C_{max} and AUC) of zoliflodacin collected up to 24 h after 2 g and 4 g dose of zoliflodacin
- Relationship between plasma concentrations of zoliflodacin (and PK and exposure parameters, such as C_{max} and AUC) and ΔΔQTcF up to 24 h after 2 g and 4 g dose of zoliflodacin
- Occurrence of treatment-emergent SAEs, other unsolicited treatment-emergent AEs, and changes from baseline in VS, clinical laboratory values, and ECG parameters following

administration of zoliflodacin and moxifloxacin from time of dosing to Final Visit, or ET Visit

4 STUDY DESIGN

The TQT study will be performed in a single center, the Vince & Associates Clinical Research, Inc., clinical trials unit (CTU), according to a randomized, double-blinded (except for the use of moxifloxacin), placebo-controlled, four-period, four-treatment, crossover design balanced with respect to first-order carryover effect in 72 healthy male or female subjects, aged 18 to 45 years inclusive, to evaluate the effect of zoliflodacin on QTcF and other ECG parameters, the correlation of the drug concentrations (and PK profile) with ΔΔQTcF, and the PK and safety profiles of the new zoliflodacin formulation.

Subjects who consent to participate will be enrolled in the trial if they meet all inclusion and no exclusion eligibility criteria. Laboratory assessments at Screening Visit will include routine clinical laboratory tests (HEM, CHEM, and UA), testing for viral serology (HIV antibody, HBsAg, and HCV antibody), 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.

Each subject will receive one dose of each of four treatments, labeled A through D:

- Treatment A: zoliflodacin 2 g PO (investigational drug, therapeutic dose)
- Treatment B: zoliflodacin 4 g PO (investigational drug, supratherapeutic dose)
- Treatment C: placebo PO (for zoliflodacin 4 g)
- Treatment D: moxifloxacin 400 mg PO (positive control comparator)

In the dosing phase, each subject will be randomly allocated to one of 12 dosing sequences before dosing on Day 1, Dosing Period 1, and receive a single dose of the following four study drugs on different days according to their randomly-assigned dosing sequence, as follows:

Table 1: Dosing Sequences

ABCD	ACDB	ADBC
BADC	BDCA	BCAD
CDAB	CABD	CBDA
DCBA	DBAC	DACB

Six of the planned 72 subjects will be included in each treatment sequence. The design is balanced for period and first-order carryover effects.

Subjects will be dosed in the morning of Day 1 in each dosing period, in a staggered fashion at least several minutes apart, and will receive study drug at approximately the same time in each

subsequent dosing period. Each treatment will be administered 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. Zoliflodacin or placebo will be reconstituted in 60 mL of tap water. After the cup containing the 60 mL of zoliflodacin or placebo is taken, approximately 60 mL of tap water will be added to the cup and consumed by the subject to chase the initial dose. Moxifloxacin will be administered orally with 120 mL of water.

Safety assessments post-dosing will include VS, PE, 12-lead standard ECG, and clinical laboratory tests including HEM, CHEM, and UA. Treatment-emergent AEs and SAEs will be assessed from time of dosing to end of trial.

Continuous 12-lead Holter ECGs will be performed on Day 1 starting approximately 1 h before dosing and lasting 25 h. At timepoints for ECG extraction (see Section 8.3.1), subjects will be resting in the supine position for at least 10 min before and 5 min after the nominal time, without any ongoing stimulation (e.g., television). Three replicate ECGs will be extracted at each timepoint to measure ECG intervals and assess T-wave morphologies.

Plasma for PK analysis will be collected at scheduled timepoints that match timepoints for ECG extraction before and after dosing on Day 1 and through Day 2, or ET if it occurs within 24 h after dosing.

Subjects will be monitored as inpatients in the CTU up to 24 h after each dose (Day 2). They will then be discharged from the inpatient CTU and followed as outpatients for at least 5 days (Days 3-7) after dosing in Dosing Periods 1-3. The total time to wash-out of the previously-received study drug is at least 8 days. The Final Visit will be 8 ±2 days after the last dose.

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 safety monitoring in subsequent clinical trials with zoliflodacin. If criteria for halting the trial (as listed in Section 9.5.1) are met in any dosing period, dosing will be suspended, and 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 principal investigator (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 no exclusion criteria will be eligible for enrollment into the trial. No exemptions are granted on Inclusion/Exclusion Criteria in DMID-sponsored trials.

72 healthy male and female subjects, aged 18 to 45 years inclusive, will be enrolled in the trial.

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 authorized site clinician (listed on FDA Form 1572)
3) Willingness to comply with and be available for all protocol procedures, including inpatient confinement for 3 days in each dosing period and follow-up for the duration of the trial
4) Aged 18 to 45 years inclusive on the day of first 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 all female subjects, whether of childbearing potential or post-menopausal by MH, a negative serum pregnancy test at Screening Visit and on Day -1 of each dosing period
    - *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 to become pregnant during the trial and for 30 days after Final Visit
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 Visit
    - *Note: A highly effective method of birth control is defined as one with a low failure rate (i.e., <1% per year) according to CDC criteria.[23] 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 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, are in the normal reference range with acceptable exceptions as noted in Section 8.2.1 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 8.2.5)

14) Urine alcohol test is negative

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

5.2 Subject Exclusion Criteria

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

1) History of acute or chronic cardiovascular disease or surgery

   - *Note: Conditions include: congestive heart failure; coronary artery disease (myocardial infarction, unstable angina); cerebrovascular disease (cerebrovascular accident or stroke or transient ischemic attack (TIA); chronic hypertension; or coronary revascularization surgery (coronary artery bypass grafting or percutaneous transluminal coronary angioplasty)*

2) History of cardiac arrhythmia or syncope related to cardiac arrhythmia or unexplained, or use of a cardiac pacemaker

   - *Note: Conditions include: atrial fibrillation, atrial flutter, or non-sustained or sustained ventricular tachycardia); use of a cardiac pacemaker; personal or family history of LQTS; or family history of sudden death*

3) History of any other 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; 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 surgery including weight loss surgery or biliary surgery

4) Major surgical interventions are not permitted within 4 weeks of first dosing and during the trial. Minor surgical interventions are not allowed within 2 weeks of first dosing and during the trial.

5) 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*

6) Active allergic symptoms to seasonal and animal allergens that are moderate to severe, affect daily activity, and require continuous treatment

7) 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*

8) 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; any characteristics that would make QT assessment unreliable, including flat T waves; or any condition deemed clinically significant by a study investigator

9) 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

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

11) Febrile illness with temperature ≥38.0°C for <7 days before dosing in each treatment period

12) Donated whole blood or blood products within 60 days before first dosing, or plans to donate or receive before Final Visit (Day 8 ±2 after last dose in dosing period 4)

- *Note: Blood products include RBCs, white blood cells (WBCs), platelets, and plasma*

13) Known allergic reactions to fluoroquinolones or to components present in the formulation or processing of zoliflodacin and moxifloxacin

14) Treatment with another investigational product within 30 days of first 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*

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

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

- *Note 1: Prohibited medications include moderate or strong CYP3A4 inducers (per 6.6) and other drugs with known risk for QT prolongation and TdP (Appendix E and 17); 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 up to 7 days before first dosing or 7 days after last dosing*
- *Note 2: Allowed medications include: oral contraceptives; H1 antihistamines; all medications approved for control of intraocular pressure including topical ophthalmic non-selective β-blockers, such as betaxolol, carteolol, levobunolol, metipranolol, and timolol; topical/ intranasal corticosteroids; nonsteroidal anti-inflammatory drugs (NSAIDS); licensed influenza vaccine during the flu season, 7 days before first dosing or 7 days after last dosing*

17) Use of non-prescription medications, vitamins, herbs, or nutritional supplements within 15 days before first dosing or planned use during the trial unless approved by the study clinician

- *Note 1: Intake of nutritional supplements, juice, and herbal preparations or other foods or beverages that may affect the various drug-metabolizing enzymes and transporters (e.g., grapefruit, grapefruit juice, grapefruit-containing beverages, apple or orange juice, vegetables from the mustard green family [e.g., kale, broccoli, watercress, collard greens, kohlrabi, Brussels sprouts, mustard], and charbroiled meats) within 7 days before dosing*
- *Note 2: Exceptions: St. John's wart is not allowed within 30 days of dosing; vitamins*

and over-the-counter (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

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

19) Smoking or use of tobacco or nicotine-containing products within 30 days before first dosing

20) Engagement in strenuous exercise within 15 days before first dosing (e.g., marathon running, long-distance cycling, weight lifting) and during the trial

21) 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

22) 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 trial

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

23) Is a CTU employee or staff member who is paid entirely or partially by the Office of Clinical Research Resources (OCRR)/NIAID contract for the DMID-funded trial

- *Note: CTU 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 and Randomization Procedures

Seventy-two 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.

Randomized sequence assignments will be generated by a statistician at the DCC for this study. Randomization will occur following admittance to the CTU and confirmation of eligibility is confirmed.

Subjects will be randomized to one of 12 treatment sequences (Section 4) with equal allocation (1:1:1:1:1:1:1:1:1:1:1:1). The randomization list will be generated centrally through the AdvantageEDC^{SM} (Electronic Data Capture [EDC] System, Emmes) by the unblinded study biostatistician, and a list will be transferred to the unblinded study pharmacist before starting the study for the purpose of an emergency back-up.

Per ICH guideline E6: GCP, screening records will be kept at the CTU to document the reason why an individual was screened but did not meet trial entry criteria, by recording it in AdvantageEDC^{SM}.

Enrollment of subjects will be done online using the enrollment module of AdvantageEDC^{SM}. The randomization code will be prepared by statisticians at the DCC and included in the enrollment module for this trial. AdvantageEDC^{SM} will assign each subject to a treatment sequence after the demographic and eligibility data have been entered into the system. A designated individual at the participating site will be provided with a code list for emergency unblinding purposes, which will be kept in a secure place. Instructions for using the enrollment module are included in the AdvantageEDC^{SM} User's Guide.

5.3.2 Masking Procedures

This is a double-blinded clinical trial with respect to administration of zoliflodacin or placebo. The study staff administering the study drug and assessing subjects will not be aware of the administered zoliflodacin or placebo. Administration of moxifloxacin will be open-label.

The SMC will review aggregate data in open session and review grouped data only in closed session.

5.3.3 Reasons for Withdrawal and Discontinuation of Study Drug Administration

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

A subject will be discontinued from the trial if any of the following 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 will 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), FDA, or DMID;
- The subject's well-being, based on the opinion of the investigator;
- The occurrence of QTcF interval prolongation >500 msec or ≥60 msec above baseline or occurrence of a symptomatic or asymptomatic ventricular tachyarrhythmia with or without QTcF prolongation;
- The occurrence of an SAE or AE warranting withdrawal.

A subject may be removed from data analysis for a dosing period during which the following events occurred, but not withdrawn from the trial (see also Section 5.3.4):

- Failure to ingest the entire 60 mL volume of zoliflodacin suspension or placebo suspension;
- Vomiting up to 5 h after dosing with zoliflodacin or placebo or moxifloxacin.

5.3.4 Handling of Withdrawals and Discontinuation of Administration

- Subjects who are withdrawn before dosing may be replaced with a subject assigned the same sequence.
- A subject who cannot ingest the entire 60 mL volume of zoliflodacin or placebo suspension or a subject who took the entire dose but vomits up to 5 h after dosing with zoliflodacin or placebo or moxifloxacin will be withdrawn from data analysis for the period when the event occurred, but will not be withdrawn from the trial and may complete the remaining treatment periods in the assigned dosing sequence.
- Subjects who ingest the entire volume of the initial 60-mL drug suspension but who do not ingest the content of the second 60-mL rinse will not be withdrawn from the trial.
- Subjects who withdraw after receiving at least one treatment in a treatment sequence will not be replaced, unless more than 16 subjects withdraw from the trial.
- Subjects who received any amount of the study drug but withdraw from the trial within 24 h after dosing will be encouraged to continue follow-up (with subjects' consent) for safety assessments and PK sample collection.
- Subjects withdrawing will be asked to complete an ET Visit if they do not wish to be followed per protocol.

5.3.5 Lost to Follow-up

If subjects 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 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 Description of Study Products

Zoliflodacin will be the investigational drug in this clinical trial and its effects will be compared to placebo matching the 4-g dose. Moxifloxacin will be the positive control (comparator) drug.

Zoliflodacin

The drug product is presented as a powder for oral suspension in a single sachet, each containing 2 g of zoliflodacin, a spiropyrimidinetrione antibacterial agent. The sachet contains both zoliflodacin and excipients. The excipient ratio for zoliflodacin is [redacted]; the excipients are detailed in the IB and Manual of Procedures (MOP) or Pharmacy Manual. Further details regarding the method of reconstitution and administration will be provided in the MOP or Pharmacy Manual.

Zoliflodacin placebo

This is composed of 4 g of the same excipients found in the drug [redacted]. The excipients are detailed in the IB and the MOP or Pharmacy Manual.

Moxifloxacin Hydrochloride

Moxifloxacin is a slightly yellow-to-yellow crystalline substance, formulated for human use as 400-mg film-coated tablets. It is a synthetic broad-spectrum, fluoroquinolone antibacterial agent that is licensed for oral administration as an antibiotic.

Moxifloxacin is available as the monohydrochloride salt of 1-cyclopropyl-7-[(S,S)-2,8-diazabicyclo [4.3.0]non-8-yl]-6-fluoro-8-methoxy-1,4-dihydro-4-oxo-3-quinoline carboxylic acid.[18] The excipients include cellulose microcrystalline, croscarmellose sodium, magnesium stearate, ferric oxide red, ferric oxide yellow, hypromellose 2910, polyethylene glycol 400, polysorbate 80, sodium stearyl fumarate, talc, silicon dioxide, and titanium dioxide.

6.1.1 Acquisition

Zoliflodacin sachets and placebo are currently stored at a Good Manufacturing Practice (GMP) facility. Upon request by DMID, they 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 drugs will be shipped from DMID-CMS to the CTU upon request and approval by DMID. Details will be provided in the protocol-specific MOP.

Moxifloxacin hydrochloride tablets, 400 mg, NDC 55111-0112-30 , will be purchased from the manufacturer, Dr. Reddy's Laboratories Limited, Bachupally – 500 090, India, and will be stored at DMID-CMS before shipment to the CTU.

6.1.2 Formulation, Packaging, and Labeling

Zoliflodacin

Zoliflodacin is presented as granules for oral suspension, 50% wt/wt, packaged in a single sachet. Each sachet contains 2 g of spray-dried zoliflodacin, [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."*

Zoliflodacin Placebo

The placebo is provided in powder form as a bulk mixture of the drug product formulation but excluding zoliflodacin.

Moxifloxacin

Moxifloxacin is available in a bottle containing 30 unit doses of film-coated tablets of moxifloxacin hydrochloride USP equivalent to 400 mg of moxifloxacin.

6.1.3 Product Storage and Stability

Zoliflodacin

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

Zoliflodacin Placebo

The placebo will be stored at 20-25°C.

Moxifloxacin

Moxifloxacin tablets will be stored at 20-25°C (68-77°F). A high moisture (humidity) storage environment will be avoided.

The clinical supplies storage area at the CTU will be monitored by its staff for temperature consistency with the acceptable storage temperature range specified in this protocol or in the Manual of Operations. Documentation of temperature monitoring will be maintained.

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

Treatment A: One sachet containing 2 g of zoliflodacin will be reconstituted in 60 mL of tap water by the unblinded pharmacist and dosed orally after an overnight fast. The dose will be administered as a mix-and-drink solution in a standardized cup as described in the MOP. After the cup containing 60 mL of zoliflodacin is taken, approximately 60 mL of tap water will be added to the cup and consumed by the subject to chase the initial dose.

Treatment B: Two sachets containing 2 g of zoliflodacin each will be reconstituted in 60 mL of tap water by the unblinded pharmacist and dosed orally after an overnight fast. The dose will be administered as a mix-and-drink solution in a standardized cup as described in the MOP. After the cup containing 60 mL of zoliflodacin suspension is taken, approximately 60 mL of tap water will be added to the cup and consumed by the subject to chase the initial dose. The second 60 mL volume is called ‘the rinse”.

Treatment C: A single dose of 4 g of placebo in powder form, whose quantity will match that in the 4-g dose of zoliflodacin, will be reconstituted in 60 mL of tap water by the unblinded pharmacist and dosed orally after an overnight fast. The dose will be administered as a mix-and-drink solution in a standardized cup as described in the MOP. After the cup containing 60 mL of placebo is taken, approximately 60 mL of tap water will be added to the cup and consumed by the subject to chase the initial dose. The second 60 mL volume is called ‘the rinse”.

Treatment D: A single, commercially-available, film-coated, 400-mg tablet of moxifloxacin hydrochloride will be administered orally with 120 mL of tap water.

Dosing, which includes ingestion of the entire 120 mL of tap water for any of the treatments (60 mL of zoliflodacin or placebo suspension and 60 mL of the rinse, or 120 mL for taking the moxifloxacin tablet), will be completed within 5 min of initial suspension for treatment A, B, and C and ingestion for treatment D by a subject. After dosing, subjects will continue to fast for an additional 4 h while water is allowed *ad lib*.

The CTU unblinded pharmacists will complete appropriate documentation such that the reconstituted materials dispensed to the CTU are clearly identified in a document suitable for auditing and monitoring. Clinical staff will also maintain a record of the volume of study drug suspension administered to and consumed by the subject.

The study drugs will be inspected for damage, contamination, discoloration, or particulate matter before use. Any study drug that fails inspection will be quarantined at appropriate temperature

and labeled 'Do Not Use' until further notice. The Site PI or responsible person will 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 CTU will receive specific instructions on how to return it to DMID CMS or destroy it on site. Refer to the protocol-specific MOP for detailed information on the preparation and administration of zoliflodacin, placebo, and moxifloxacin.

6.3 Modification of the Study Intervention/ Investigational Products for a Subject

Not applicable for single-dose study drug trial. 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 will be reported; if the overdose is associated with an AE, then the AE will also be reported. In the event of an overdose of zoliflodacin, the investigator will use clinical judgment in treating the overdose and contact the DMID Medical Monitor (MM). The investigator will 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 Products

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

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

Upon completion of the trial and after the final monitoring visit, any remaining unused study drugs will either be returned or destroyed appropriately at the CTU as per DMID requirements and instructions that will be communicated to the CTU by the DMID CPM.

6.5 Assessment of Subject Compliance with the Study Intervention/ Investigational Products

Since each dose of zoliflodacin, placebo or moxifloxacin 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 60-mL zoliflodacin or placebo suspension or the moxifloxacin tablet, or who vomit up to 5 h after dosing with zoliflodacin, placebo or moxifloxacin will be withdrawn from data analysis for the period when the event occurred, but will not be withdrawn from the trial and may complete the remaining treatment periods in the assigned dosing sequence. Subjects who do not ingest the content of the second 60-mL rinse will not be withdrawn from the trial.

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 first dosing will be reported as Prior Medications or Concomitant Medications (ConMeds), respectively.

Prior prescription medications will be recorded at Screening Visit and will not be taken for 30 days before dosing or during the trial. ***Exceptions*** include: oral contraceptives; H1 antihistamines; all medications approved for control of intraocular pressure including topical ophthalmic non-selective β-blockers, such as betaxolol, carteolol, levobunolol, metipranolol, and timolol; topical/ intranasal corticosteroids; NSAIDS; licensed influenza vaccine during the flu season, 7 days before the first dose or 7 days after the last dose.

Zoliflodacin will not be co-administered in subjects taking medications that are strong (e.g., avasimibe, carbamazepine, phenytoin, rifampin, and St. John's wort) or moderate (e.g., efavirenz, bosentan, etravirine, modafinil, and nafcillin) inducers of CYP3A4. These medications will not be taken for 30 days before dosing and during the trial.

Zoliflodacin will not be co-administered in subjects taking medications that pose a risk of TdP in association with prolonged QT/QTc (Appendix E and 17). These medications will not be taken for 30 days before dosing and during the trial.

Moxifloxacin will not be co-administered in subjects taking multivalent cation-containing products including antacids, sucralfate, and multivitamins.

A **vaccine** will not be received within 30 days before dosing or during the trial, except for licensed influenza vaccine during the flu season, which may be administered up to 7 days before the first dose or 7 days after the last dose.

Non-prescription medications, herbs, vitamins, and nutritional supplements will not be taken within 15 days before dosing and during the trial. ***Exceptions*** include: (1) nutritional supplements, juice, and herbal preparations or other foods or beverages that may affect various drug-metabolizing enzymes and transporters (e.g., grapefruit, grapefruit juice, grapefruit-containing beverages, apple or orange juice, vegetables from the mustard green family [e.g., kale, broccoli, watercress, collard greens, kohlrabi, Brussels sprouts, mustard], and charbroiled meats) within 7 days before dosing; (2) St. John's wart within 30 days before dosing and during the trial; and (3) vitamins and OTC medications taken for <48 h for the treatment of common symptoms (e.g., headache, indigestion, muscle pain) may be allowed if approved by the designated study clinician.

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

Following dosing, each new ConMed and changes to existing medications will be recorded. Subjects will be required not to utilize non-study medications 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 vaccines 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 first 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 dosing period of the trial. Subjects will fast for at least 8 h before dosing and 4 h after dosing. Water can be taken *ad lib* during those periods. For clinical laboratory blood draws, subjects will fast for at least 4 h. Meals during each dosing period will be standardized. Otherwise, there will be no restrictions regarding food and fluid intake.

6.7.3 Alcohol, Marijuana and Illicit Drugs

Alcohol will not be used at least 3 days before first study drug administration and during the trial. Marijuana and illicit drugs are exclusionary if detected before first dosing and prohibited during the trial.

6.7.4 Other Restrictions

Smoking or use of tobacco or nicotine-containing products is prohibited within 30 days before first dosing and during the trial. Caffeinated beverages or foods containing caffeine are prohibited within 3 days before first 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 population surrounding the CTU, utilizing the CTU subject database and IRB-approved advertisements and social media. IRB-approved, prescreening 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 confirm 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 [m2])
- 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 urine 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
- Counsel to avoid rigorous physical activity

Subjects who meet the eligibility criteria will be contacted by CTU 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 HIV, HBV, and HCV will be informed of the findings and counseled to seek medical care for further evaluation and treatment. If subjects test positive for HIV antibody, HBsAg, and/or HCV antibody, they will be informed that test results may be reported to the local health authorities according to state or local law. Subjects who fail screening will not be re-screened unless they had an inter-current, short-term medical illness. Subjects who meet eligibility criteria but could not be enrolled within permissible window may be rescreened.

7.3 Check-in / Baseline (Day-1 in each dosing period)
(Overall, Days -1, 8, 16, and 24 for dosing periods 1, 2, 3 and 4, respectively)

Subjects meeting all inclusion and no 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 confirm the subject remains eligible for enrollment
- Update MH (Day -1 in Period 1 only)
- Update Prior Medications
- Perform abbreviated PE (Perform on each check-in. In Period 1, do not perform only if the screening PE was completed ≤7 days from dosing.)
- Obtain VS
- Obtain weight
- Obtain blood and urine samples for clinical laboratory tests (collect on each check-in). In Period 1, do not collect if screening samples were collected ≤7 days 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 urine alcohol test for alcohol use
- Obtain 12-lead standard ECG with 10-sec rhythm strip
- Counsel to avoid rigorous physical activity
- Review AEs/SAEs (Day -1 in Periods 2, 3, and 4, respectively, on study Days 8, 16, and 24)

Subjects who still meet the eligibility criteria on Day -1 before Period 1 will be admitted to the CTU on the same day.

For subsequent dosing periods (2, 3, and 4), subjects will be admitted on Day -1. Any new symptoms or abnormal assessments will be reviewed prior to dosing, and recorded as AEs.

Eligibility for continuation of dosing (dose 2, 3 and 4):

Dosing for subsequent periods may proceed if:

- A negative serum pregnancy test on Day -1 of each dosing period
- Females of childbearing potential and males still agree to use acceptable contraception for the duration of the trial and for 30 days (females) or 90 days (males) after Final Visit
- One of the following:
  - Subjects who did not experience any AE since the last dose administration
  - Any new or prior AEs were grade 1 or resolved, and in the opinion of the site principal investigator or appropriate sub-investigator, the AEs, individually or in cluster, do not pose a risk to the subject or would be likely to confound interpretation of the results.
  - Any new or prior AEs that were grade 2, should be either resolved or downgraded to Grade 1 and in the opinion of the site principal investigator or appropriate sub-investigator, the AEs, individually or in cluster do not pose a risk to the subject or would be likely to confound interpretation of the results.
  - Any new or prior grade 3 AEs that were judged unrelated to study drug should be either resolved or downgraded to Grade 1 and in the opinion of the site principal investigator or appropriate sub-investigator, the AEs, individually or in cluster do not pose a risk to the subject or would be likely to confound interpretation of the results.

If a subject has a Grade 3 AE judged related to study drug administration, this subject will be withdrawn from the study and will not receive subsequent dosing. For all other AEs, dosing may be delayed up to 14 days in order to follow the AE for downgrading or resolution according to the above. If the subject does not meet the criteria for dosing specified above in 14 days, this subject will be withdrawn from the study and will not receive subsequent dosing.

After admission to the CTU, the subject will fast at least 8 h before dosing but will have access to water during that period.

7.4 Inpatient period (Days 1-2 in each dosing period) (Overall, Days 1-2, 9-10, 17-18, and 25-26 for dosing periods 1, 2, 3 and 4, respectively)

7.4.1 Randomization (Day 1, Dosing Period 1 only)

A subject who continues to meet eligibility criteria by assessments performed before dosing will be enrolled in the trial and randomized on Day 1 of Dosing Period 1 to one of the 12 study treatment sequences.

7.4.2 Administration of Study Drug (Day 1 of each dosing period) (Overall, Days 1, 9, 17, and 25 for dosing periods 1, 2, 3 and 4, respectively)

Before Dosing:

- Withhold breakfast but allow access to water
- Review inclusion/exclusion criteria to confirm the subject remains eligible for dosing

- Update MH (Period 1) / AE review (Periods 2, 3, and 4)
- Update Prior Medications /Document concomitant medications (ConMeds) (Periods 2, 3, and 4)
- Obtain VS within 60 min before dosing
- Perform symptom-directed PE, if applicable
- Obtain 12-lead standard 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 within 30 min before dosing
- Initiate 12-lead Holter ECG at least 1 h before dosing
    - Subjects will remain in a supine position at rest for at least 10 min before and 5 min after each of the following timepoints: 45, 30, and 15 min before dosing.
    - ***If timepoints for ECG extraction coincide with 12-lead standard ECGs, VS assessments, and PK blood draws, these procedures will be performed in this order: 12-lead Holter ECG, 12-lead standard ECG, VS, and PK blood draw.***

Dosing:

- Administer a single oral dose of the study drug with water, as described in Section 6.2, according to the randomization schedule for each treatment sequence and period
- Withhold food until 4 h after dosing but allow access to water

After Dosing:

- Continue 12-lead Holter ECG:
    - Subjects will remain in a supine position at rest for at least 10 min before and 5 min after each of the following timepoints: 0.5, 1, 2, 3, 4, 6, 8, and 12 h after dosing.
    - ***If timepoints for ECG extraction coincide with 12-lead standard ECGs, VS assessments, and PK blood draws, these procedures will be performed in this order: 12-lead Holter ECG, 12-lead standard ECG, VS, and PK blood draw.***
- Obtain 12-lead standard ECG with 10-sec rhythm strip at 1 h (±10 min), 2 h (±10 min), and 4 h (±10 min) 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 status
- 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
- Perform symptom-directed PE, if applicable
- Perform AE and SAE assessments
- Document ConMeds

7.4.3 Inpatient Follow-up (Day 2 of each dosing period)
(Overall, Days 2, 10, 18, and 26 for dosing periods 1, 2 3 and 4, respectively)

- Continue 12-lead Holter ECG:
  - Subjects will remain in a supine position at rest for at least 10 min before and 5 min after the following timepoint: 24 h (±2 h) after dosing.
  - ***If the timepoint for ECG extraction coincides with 12-lead standard ECG, VS assessment, and PK blood draw, these procedures will be performed in this order: 12-lead Holter ECG, 12-lead standard ECG, VS, and PK blood draw.***
- Obtain VS
- Perform symptom-directed PE, if applicable
- Perform AE and SAE assessments
- Obtain clinical labs before discharge
- Obtain blood (plasma) PK samples at 24 h (±2 h) after dosing
- Document ConMeds
- Obtain 12-lead standard ECG at 24 h (± 2 h) after dosing (before discharge on Day 2)
- Remove 12-lead Holter recorder on Day 2 at 24 h after dosing after last recording
- Remind women of childbearing potential on the avoidance of pregnancy
- Remind male and female subjects on the use of appropriate contraception
- Remind male subjects to refrain from sperm donation
- Remind subjects to abstain from prohibited medications, alcohol, marijuana, and illegal drugs
- Remind subjects to abstain from rigorous physical activity
- Instruct on the next scheduled visit
- Discharge subject from the CTU after review of clinical laboratory tests, ECGs, and other assessments by PI or authorized clinician

7.5 Outpatient period (Days 3-7 for each dosing period
(Overall, Days 3-7, 11-15, 19-23 and 27-31 for dosing periods 1, 2, 3 and 4, respectively)

This will be a 5-day outpatient period between completion of treatment in the previous period and re-admission to the CTU for next dosing period except after Dose 4. Subjects will report to the CTU any new AEs/SAEs they have experienced, and medications they have used during the outpatient periods.

7.6 Final Visit (Day 32, 8 ±2 days after last dose in Dosing Period 4)
- 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
- Obtain 12-lead standard ECG with 10-sec rhythm strip
- Obtain blood and urine for clinical laboratory tests
- Remind male and female subjects on the avoidance of pregnancy
- Remind female subjects to use appropriate contraception for 30 days after Final Visit
- Remind male subjects to use appropriate contraception and refrain from sperm donation for 90 days after Final Visit
- Discharge subject from the trial

7.7 Early Termination Visit (if needed)

- Obtain VS
- Perform complete PE
- Perform AE and SAE assessments
- Update ConMeds
- Obtain 12-lead standard ECG with 10-sec rhythm strip
- Obtain blood and urine for clinical laboratory tests
- Collect blood for serum β-HCG pregnancy test in all females
- Obtain blood PK sample if ET occurs within 24 h of dosing
- Remind male and female subjects on the avoidance of pregnancy
- Remind female subjects to use appropriate contraception for 30 days after ET
- Remind male subjects to use appropriate contraception and refrain from sperm donation for 90 days after ET

7.8 Unscheduled Visit (if needed)

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

- 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
- Obtain 12-lead standard ECG with 10-sec rhythm strip as needed for the evaluation of an AE
- Remind 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 will 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 first study drug dosing and will be documented on the subject's source documents and eCRF. Confirmation of eligibility will be performed before dosing on Day 1 of each dosing period.

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 of 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 cardiovascular disorders, including MI and angina, cardiac arrhythmias including LQTS, syncope due to cardiac arrhythmias or unexplained, psychiatric illness, and substance abuse will be specifically solicited. Family history especially of sudden death will be obtained. The MH will be obtained at Screening Visit and updated upon admission to the CTU and before dosing on Day 1 in Period 1. After first study drug dosing, 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 after Dosing Period 4), or ET Visit, 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 in each dosing period on Day -1 in each inpatient period. If a complete PE was performed ≤7 days before first dosing, then an abbreviated PE will not be performed on Day -1 of the Dosing Period 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 in each dosing period before dosing on Day 1 and after dosing on Days 1 and 2, 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 of each dosing period and at Final Visit (Day 8 ±2 after Dose 4) or ET.

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 5 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, and on Day 2 of each of the four dosing periods, and at Final Visit after Dose 4, or ET. On Day 1 of each dosing period, 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 Day 2, VS will be measured at 24 h (±2 h) after dosing. Acceptable ranges are shown in Appendix B.

VS that are considered aberrant due to an error in measurement may be repeated. At 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 12-lead Standard Electrocardiogram (ECG)

A 12-lead standard ECG and 10-sec rhythm strip will be obtained at Screening Visit and on Day -1, Day 1 before and after dosing, Day 2 (24 ±2 h after dosing) in each treatment period, and Final Visit after Dose 4, or ET. On Day 1 of each dosing period, a 12-lead standard 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 on 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 HEM and CHEM tests will be approximately 9 mL per sample (Appendix D).

8.2.1 Clinical Laboratory Evaluations (Hematology, Chemistry, and Urinalysis)

Blood and urine samples for clinical laboratory tests will be collected at Screening Visit, on Day -1 of each dosing period (with the exception in the first dosing period if screening clinical labs were collected ≤7 days of Dose 1), on Day 2 of each dosing period, and Final Visit after Dose 4, or ET. Subjects must be fasting for > 4 h before any blood draw for clinical laboratory assessments. These tests will include:

- HEM: Hgb, Hct, RBC count, platelet count, and WBC count with absolute differential count
- 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, total carbon dioxide (CO_2), magnesium
- 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 of Treatment Period 1 should be in the normal reference range with acceptable exceptions, as shown below and in Appendix B:

Elevated bilirubin due to documented Gilbert 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 two 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 values that reach toxicity Grade 1 values are 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 after 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 to AdvantageEDC℠.

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.

8.2.2 Viral Serology Testing

Serological testing for HIV antibody, HBsAg, and HCV antibody 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.

8.2.3 Pregnancy Testing

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

8.2.4 Serum FSH Testing

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

8.2.5 Urine Toxicology Screening

A urine toxicology screen will be performed at Screening Visit and on Day -1 to detect the presence of amphetamines, cocaine (and metabolite), barbiturates, benzodiazepines, opiates, MDMA, methadone, tetrahydrocannabinol, methamphetamines, tricyclic antidepressants (TCAs), 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.

8.2.6 Urine Alcohol Testing

Detection of recent alcohol consumption in urine will be performed at Screening Visit and on Day -1 in each treatment period. Results must be negative for study eligibility.

8.3 Special Procedures and Assays

8.3.1 Continuous 24-h ECG Monitoring (Holter ECG Monitoring)

ECGs for assessment of the primary endpoint will be obtained digitally during all four dosing periods using a continuous 12-lead Holter ECG digital recorder system. The continuous ECG recording will be started approximately 1 h before dosing on Day 1 of all dosing periods and will continue until 24 h after dosing (i.e., in the morning of Day 2). Three replicate ECG recordings will be extracted at the following timepoints in each dosing period: 45, 30, and 15 min before dosing (baseline) and 0.5, 1, 2, 3, 4, 6, 8, 12, and 24 h after dosing for a total of 12 timepoints per subject per dosing period. The subject will be in a supine position for at least 10 min before recording an ECG and 5 min after the nominal timepoint for the ECG extractions from the Holter monitors. These timepoints will correspond to PK blood sampling for each dosing period, but ECGs will be obtained before the measurement of VS and collection of blood samples, to avoid the effect of stress on the ECG. The mean of pre-dose ECGs extracted on Day 1 of each dosing period will be used as baseline reference for results obtained after dosing.

ECGs will be extracted from the Holter recordings over a 5-min period and used to measure ECG intervals. Digital QT, QTcF, PR, QRS, and RR intervals and HR will be determined by the Core ECG Lab. Digital ECGs will be read in accordance with the principles outlined in ICH E14 Guidance for TQT studies.[16]

The 12-lead Holter and ECG equipment will be supplied and supported by the VACR Phase 1 CTU. All ECG data will be collected using a continuous 12-lead ECG digital recorder. The continuous 12-lead digital ECG data will be stored onto secure digital memory cards. ECGs to be analyzed will be collected at pre-determined timepoints (as indicated above and in Appendix A: Schedule of Study Procedures and Evaluations) and will be read centrally by IQVIA Cardiac Safety Services.

The following principles will be followed in the IQVIA Cardiac Safety Services core laboratory:

- Each extracted ECG will be analyzed by the cardiologist who will be blinded to subject identifiers, time, and treatment.
- Electrocardiograms will be analyzed for PR, QRS, RR, and QT intervals using semi-automated measurements and for morphological abnormalities including T and U waves manually by experienced cardiologists.
- A limited number of skilled readers will be employed in the over-read process to control for variability in interpretation.
- All ECGs from a single subject will be read together by the same reader.

- Consistent leads will be used for estimation of ECG intervals to the extent possible, with lead II as the primary lead. If technical issues prevent measurement in lead II, the QT will be measured in an alternative lead as per the core laboratory procedure.

The following sections provide a brief description of ECG analysis methods utilized by the core laboratory.

8.3.1.1 TQT and ECG Extraction Technique

Three replicate ECG recordings will be extracted at the indicated timepoints from each of the four dosing periods. For each timepoint, ECG extraction will typically be from the last 5 min of the period (at least 10 min) when the subject is maintained in a supine quiet position before the nominal timepoint (i.e., 25-30 min for the 0.5-h post-dosing timepoint and 55-60 min for the 1-h post-dosing timepoint).

The ECG Core Lab will use Antares software to extract ECGs from the continuous 24-h Holter recordings after evaluating the complexes for sinus origin, heart rate stability criteria and the smallest noise score. During protocol-specified ECG extraction windows, three 10-sec digital 12-lead ECG tracings will be extracted from continuous Holter recordings. The mean QT, QTcF, and RR values from each extracted replicate will be calculated, and the average of all mean values of primary ECG parameters from all replicate ECGs from a nominal timepoint will be used as the subject's reportable value at that timepoint.

The IQVIA Cardiac Safety Services will submit extracted ECG files in FDA-compliant HL 7 XML to the FDA ECG warehouse.

8.3.1.2 T-wave Morphology Analysis and Measurement of PR and QRS Intervals

Categorical T-wave morphology analysis will be performed manually in the three ECG replicates at each timepoint. Each fiducial point (onset of P-wave, onset of Q-wave, offset of S-wave, and offset of T-wave) will be electronically marked.

The original ECG waveform (**Table 2**) and such annotations will be saved separately at the conclusion of the trial in Extensible Markup Language (XML) format for independent review and upload to the FDA ECG Warehouse.

Table 2: T-wave Morphology Categories (Assessed Manually)

Category	Description
Normal T-wave	Any T-wave not meeting any criterion below
Flat T-waves	T amplitude <1 mm (either positive or negative) including flat isoelectric line
Notched T-wave (+)	Presence of notch(es) of at least 0.05 millivolt (mV) amplitude on ascending or descending arm of the positive T-wave
Biphasic	T-wave that contains a second component with an opposite phase that is at least 0.1 mV deep (both positive and negative/positive and polyphasic T-waves included)
Normal T-wave (-)	T amplitude that is negative, without biphasic T-wave or notches
Notched T-wave (-)	Presence of notch(es) of at least 0.05 mV amplitude on descending or ascending arm of the negative T-wave

In addition to the T-wave categorical analysis, the presence of abnormal U waves will be noted. However, discrete U waves will be excluded from QT/QTc interval measurements.

8.3.2 Pharmacokinetics

8.3.2.1 Assay for Zoliflodacin

Blood (plasma) samples for assay of zoliflodacin PK will be collected in K_2EDTA tubes at the following study days and timepoints: On Day 1 within 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; and on Day 2 at 24 h (± 2 h) after dosing, or ET if occurs within 24 h of dosing.

Blood samples will be obtained promptly after each corresponding Holter recording and 12-lead standard ECG timepoint and VS assessment.

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

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

8.3.2.2 Assays for Placebo and Moxifloxacin Samples

Blood samples obtained during placebo dosing periods will not be analyzed. Blood samples obtained during moxifloxacin dosing periods will be stored and analyzed only for-cause.

8.3.3 Specimen Preparation, Handling, and Shipping

8.3.3.1 Instructions for Specimens Preparation, Handling, and Storage

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

Blood samples left after all routine clinical laboratory testing and pharmacokinetic assays are completed will not be stored indefinitely or used for purposes other than those described. No genetic testing will be done on blood samples collected.

8.3.3.2 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 DMID-CMS 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

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

KCAS Bioanalytical Services
c/o Marsha Luna, Senior Manager, PBI
12400 Shawnee Mission Parkway
Shawnee, KS 66216
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 DMID of SAEs within 24 h of site awareness;
- Provide detailed written reports, including necessary documentation requested by DMID 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, causality, and severity of:

1. Treatment-emergent SAEs occurring from time of first dose in dosing period 1 through Final Visit (Day 8 ±2 after last dose in dosing period 4), or ET;
2. Clinical laboratory AEs occurring from time of first dose in dosing period 1 through Final Visit (Day 8 ±2 after last dose in dosing period 4), or ET;
3. Non-serious, unsolicited treatment-emergent AEs occurring from time of first dose in dosing period 1 through Final Visit (Day 8 ±2 after last dose in dosing period 4), or ET.

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

9.2.1 Adverse Events

Definitions

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 will be considered a baseline finding and not reported as an AE. However, if the condition increases in severity or frequency at any time during the trial, it will be recorded as an AE.

All AEs will 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.

9.2.1.1.1 Relationship to Study Products

AEs and SAEs will 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
- **Not Related**: There is not a reasonable possibility that the study drug caused the AE/SAE

The investigator will 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.

9.2.1.2 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 and relationship to the study drug, date of resolution of the event, seriousness, and outcome.

All AEs will be documented from the time of study drug dosing through 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 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.

  *An AE is considered "life-threatening" if, in the view of either the investigator or DMID, 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.

9.2.3 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 determine 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 after dosing 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 first 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 Grade 1 abnormalities, will be evaluated by the CTU clinician and reported as Grade 1 abnormalities if clinically significant. If not clinically significant, these will not be considered laboratory AEs and thus will not be graded but will be recorded in the source document and followed-up clinically at the discretion of the CTU clinician. Abnormal laboratory

values, performed as part of HEM, CHEM, 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 menses 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 or ET Visit

Any AE that meets a protocol-defined serious criterion will 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 will 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 will be provided as soon as possible.

The CTU will copy the ISM on SAE reports provided to the DMID PVG. The DMID Medical Monitor (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 subject safety and protocol conduct.

At any time after completion of the trial, if the Site PI or appropriate sub-investigator becomes aware of an SAE that is suspected to be related to study drug, 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 DMID 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 DMID determines that the information qualifies for reporting as specified in 21 CFR Part 312.32. DMID will also notify FDA of any unexpected fatal AE or suspected life-threatening AE as soon as possible, but in no case later than 7 calendar days after DMID'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 will be reported; if the overdose is associated with an AE, then the AE will also be reported. In the event of an overdose of study drug, the investigator will use clinical judgment in treating the overdose and contact the DMID MM. The investigator will 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 trial 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 Final Visit. A male subject who participates in the trial and whose female partner becomes pregnant from the time of first dosing to 90 days after Final 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

9.5.1.1 Halting Criteria for Zoliflodacin and Placebo Periods

Study dosing of zoliflodacin or placebo will be temporary halted, and a review of available safety data will be conducted by the SMC if any of the following occur after dosing:

1. Death of a subject following dosing regardless of relatedness to the study drug;
2. One subject with a non-fatal SAE that is considered related to the study drug by the study PI or authorized physician (listed in FDA Form 1572);
3. Two or more subjects experience a QTcF >500 msec or ≥60 msec above baseline, based on readings from at least two standard 12-lead ECGs after study drug administration;
4. 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;

***Note: VS abnormalities will be considered part of a systemic disorder or an organ-specific condition, as described in* Appendix C: Adverse Events Toxicity Grading Criteria**

, in order to be included among the Study Halting Criteria.

5. One subject experiences a symptomatic ventricular tachyarrhythmia with or without QTcF prolongation after study drug administration;
6. An overall pattern of symptomatic, clinical non-serious events 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 a subject in the trial.

9.5.1.2 Halting Criteria for Moxifloxacin Periods

Study dosing of moxifloxacin will be temporary halted, and a review of available safety data will be conducted by the SMC if any of the following occur after dosing:

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

Note: VS abnormalities will be considered part of a systemic disorder or an organ specific condition, as described in Appendix C**: Adverse Events Toxicity Grading Criteria**
, in order to be included among the Study Halting Criteria.

9.6 Safety Oversight

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 will 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 cumulative, unblinded interim safety data when available after database lock

Procedures for SMC reviews/meetings will be defined in the SMC charter. The SMC will review safety data and advise on resumption or stopping of a temporary halted trial, and safety monitoring in future clinical trials with zoliflodacin.

10 CLINICAL MONITORING

10.1 Site Monitoring Plan

Site monitoring will be conducted to ensure that human subject protections, study and laboratory procedures, study intervention administration, and data collection processes are of high quality and meet DMID, 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, subjects' source documents, eCRFs, ICFs, clinical and laboratory reports, and protocol and GCP compliance. Site monitors will have access to the CTU, 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

The trial will evaluate the following hypotheses:

a. Zoliflodacin administered at 2-g or 4-g doses causes a prolongation of the QTcF interval that exceeds the criteria in the ICH Guidance E14.[16]

   (A negative effect is defined in ICH Guidance E14 as "one in which the upper bound of the one-sided 95% confidence interval (CI) for the largest time-matched mean effect of the drug on the QTcF interval is <10 msec.")

b. The trial has the sensitivity to detect an effect on QTcF of regulatory concern, as produced by moxifloxacin.

   (Assay sensitivity will be demonstrated if, for at least one individual timepoint between 1h and 4h after administration of moxifloxacin, the lower bound of the one-sided 95% CI for ΔΔQTcF, is >5 msec.)

11.2 Sample Size Considerations

Enrollment of 72 subjects would provide at least 84% power to conclude a negative effect, given that up to 16 subjects may withdraw early prior to beginning to replace subjects (at least 56 subjects evaluable), and assuming a standard deviation of ΔΔQTcF of 7 msec and an underlying effect of 5 msec.

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 advise on safety monitoring in future clinical trials of zoliflodacin. If criteria for halting the trial (as listed in Section 9.5.1) are met, 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 unblinded statistician may present data in a closed session of an SMC meeting.

11.4 Final Analysis Plan

The ICH Guidance E9 (Statistical Principles for Clinical Trials) will be followed for all statistical content.[24] Summaries of frequencies and percentages will be presented for categorical data, and summary statistics such as minimum, median, mean, SD, and maximum values will be presented for continuous data. Details of safety, QTcF, other ECG interval measurements (QT, PR, QRS, RR and HR [based on RR measurements]), T-wave changes, ECG morphology, and PK and

PK/PD data analyses and presentations will be described in the Statistical Analysis Plan (SAP) and accompanying Tables, Listings, and Figures (TLF) templates. The SAP for safety and PK data will be prepared and finalized by the DCC before final data lock. The SAP for Holter ECG data will be prepared by IQVIA Cardiac Safety Services (CSS) and appended to the SAP. Analyses included in the clinical study report (CSR) will be performed by the DCC and CSS after final data lock. Any change from originally planned statistical analyses will be reported in the CSR.

11.4.1 Analysis Populations

All subjects who received at least one dose of study drug (zoliflodacin, placebo, or moxifloxacin) 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. If any subjects are found to be noncompliant with respect to dosing or have incomplete data, a decision to include them in the analysis will be made on a case-by-case basis.

The PD analysis population will include all subjects who provide evaluable Holter ECG data from at least one study treatment, without protocol deviations potentially impacting the ECG intervals. Exclusions from the PD analysis population due to protocol deviations will be decided before unblinding. Subjects must have at least one ΔΔQTcF value defined for inclusion in the PD analysis population.

The PD and PK analysis population will include all subjects who provide both evaluable Holter ECG and zoliflodacin PK data for the relevant study treatment. The PK/QTcF population will be the subset of subjects included in both the PD analysis population and the PK analysis population. Additionally, for the concentration-QTcF analysis, time-matched zoliflodacin concentrations corresponding to the ECG assessments will also be necessary.

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

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, SD, median, minimum, and maximum) for clinical laboratory data, 12-lead standard ECG and Holter 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 (with the exception of Holter ECG, see below). For change-from-screening summaries, subjects with an undefined change from screening, due to missing data, will be excluded. Clinical significance of abnormalities will be indicated in the listings.

11.4.4 PK Analysis

PK parameters will be estimated for zoliflodacin by noncompartmental methods using version 8.0 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.

The results will be listed by subject, and summarized with descriptive statistics including: n, mean, SD, coefficient of variation, median, minimum, maximum, geometric mean, and geometric SD.

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.

11.4.6 Analysis of Continuous ECG Parameters (Pharmacodynamics)

11.4.6.1 ECG Analysis and Interpretation

The ΔΔQTcF, calculated as differences between time-matched mean change-from-baseline QTcF values for active treatment relative to placebo, will be evaluated to quantify the QTcF prolongation of the active treatments. The median of the three Holter ECG QTcF interval measurements at 45, 30, and 15 min before each dosing will be regarded as baseline for the respective period.

QTcF corrections will be calculated using Fridericia's formula:

- $QTcF=QT/RR^{0.33}$

The primary variable will be the change-from-baseline QTcF (ΔQTcF). Other endpoints to be evaluated include HR (bpm; calculated from RR interval measurements), PR (msec), QRS (msec), RR (msec), and cardiac abnormalities (morphologies, rhythm, and conduction).

All statistical analyses and reporting of digital ECG data will be conducted on the mean of the replicate intervals within a timepoint. Descriptive statistics including two-sided 90% CI will be generated for observed, change-from-baseline, and time-matched placebo-corrected change-from-baseline ECG intervals.

11.4.6.2 Primary Analysis: QTcF Effect

The primary hypothesis to be tested is that following administration of zoliflodacin 2 g and 4 g, the upper bound of the one-sided 95% CI of treatment effect on ΔΔQTcF is ≥10 msec for at least one of the ECG assessments, against the alternative hypothesis that all mean effects are <10 msec.

The primary analyses will be conducted to evaluate the effects of zoliflodacin 2 g and 4 g on cardiac repolarization as measured by ΔQTcF relative to placebo. Analysis will be performed using a linear mixed model with ΔΔQTcF as the dependent variable, with baseline QTcF, sequence, period, treatment, time (categorical), and interactions of time with treatment as fixed

effects, and subject nested within sequence as random effects. At each of the 9 post-baseline timepoints and for each zoliflodacin dose, a one-sided 95% CI for the treatment effect on ΔΔQTcF will be estimated using least square means. There will be 18 CIs, one for each combination of zoliflodacin dose and timepoint. A negative effect of 2 g or 4 g zoliflodacin will be concluded if the maximum of the 18 one-sided 95% CI upper bound is <10 msec. No adjustment will be made for multiplicity, since it is not required under intersection-union testing, as planned for the trial. No subgroup analyses are planned.

11.4.6.3 Secondary Analyses: Non-QTc Intervals

Non-QTc intervals (PR, QRS, and RR intervals) and HR (estimated from measurement of RR intervals) will be tabulated at each timepoint for each treatment group and the mean ± SD, median, 95% CI, minimum, and maximum values will be shown. Comparisons among treatment groups will be done using linear mixed effects models.

Categorical analysis of PR, QRS, and RR intervals will also be performed. The following criteria will be used to specify noteworthy non-QTc intervals (PR, QRS, and RR intervals) and HR (derived from RR intervals) and the number of subjects and number of timepoints will be tabulated for each treatment group and may be compared statistically:

- PR change-from-baseline >25% resulting in PR >200 msec
- QRS change-from-baseline >25% resulting in QRS >120 msec
- HR change-from-baseline >25% decrease resulting in HR <50 bpm
- HR change-from-baseline >25% increase resulting in HR >100 bpm

11.4.6.4 Assay Sensitivity

Another secondary hypothesis is that a moderate increase in QTcF with administration of the positive control moxifloxacin 400 mg is detectable in this trial.

The model as described in the primary analyses will be repeated with the moxifloxacin treatment for assay sensitivity evaluation. The 1, 2, 3, and 4 h timepoints will be evaluated for assay sensitivity. Type I error (familywise error rate) will be controlled at an overall level of 5% by using a prespecified multiplicity adjustment. Assay sensitivity will be demonstrated if the lower bound of one-sided 95% CI (bounds will vary based on multiplicity adjustment) for treatment effect on ΔΔQTcF in at least one timepoint is >5 msec. In addition, two-sided 90% CIs will be obtained for the treatment effect on ΔΔQTcF at every timepoint and displayed graphically.

11.4.6.5 Categorical Analyses

QTcF intervals will be categorized and tabulated for each treatment. The following criteria will be used to specify noteworthy QTcF intervals:

- QTcF >450 msec
- QTcF >480 msec

- QTcF >500 msec
- QTcF increase ≥30 msec from baseline
- QTcF increase ≥60 msec from baseline

The number and percent of subjects in each category will be calculated. Comparisons across treatments may be conducted if sufficient counts are observed.

11.4.6.6 Cardiac Abnormalities:

The morphological waveform analysis of the ECG data for TQT evaluations at each timepoint will be utilized for the summary of new onset cardiac abnormalities for each treatment. Results will be summarized in frequency tables with counts and percentages for both number of subjects and number of timepoints.

11.4.7 Concentration-QTcF Modeling

Concentration-QTcF modeling will be utilized to characterize the exposure-QTcF relationship for zoliflodacin.[25,26] Initially, linear or non-linear mixed-effect models will be fit to ΔΔQTcF values for only the zoliflodacin arms. More complex models will be employed if simpler models provide inadequate model fit. Hysteresis effects will be examined and may be utilized to characterize the delayed effects if observed. Estimates of the mean prolongation at the geometric mean zoliflodacin C_{max} for the zoliflodacin 2-g and 4-g doses will be made from the final exposure response model with corresponding two-sided 90% CIs.

Detailed analysis plans will be documented in the SAP. Results of the concentration-QTcF evaluations will be reported in the CSR.

12 SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA/DOCUMENTS

The CTU 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 CTU will permit authorized representatives of 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 CTU. 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 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 DMID, 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 CTU for clarification and resolution.

14 ETHICS/PROTECTION OF HUMAN SUBJECTS

14.1 Ethical Standard

The PI will ensure that the trial is conducted in full conformity with principles of the Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research of the National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research (April 18, 1979) and codified in 45 CFR 46, 21 CFR 50 and 56, and ICH E6; 62 Federal Regulations 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 associated ICFs by an appropriate IRB/EC listed on the FWA. Any amendments to the protocol or consent materials will 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 DMID will 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 will be obtained before any protocol-specified procedures or interventions are carried out, and in accordance with 21 CFR 50.25 and 45 CFR 46. Information will 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 will 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 will also be informed of alternative procedures that may be available, and the important potential benefits and risks of these available alternative procedures. Subjects will 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 will 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 will 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. Subjects will 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, will coerce or unduly influence a subject to participate or continue to participate in the trial. The extent of the confidentiality of the subjects' records will be defined, and subjects will be informed that applicable data protection legislation will be followed. Subjects will be informed that the monitor(s), auditor(s), IRB, DMID, 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 subject's confidentiality, to the extent permitted by applicable laws and regulations, and that, by signing a written ICF, the subject is authorizing such access. Subjects will 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 will 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 will be kept on file by the investigator for possible inspection by regulatory authorities and/or DMID and regulatory compliance persons. The subject will receive a copy of the signed and dated written ICF and any other written information provided to the subjects and will 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 aged <18 years will be excluded from participation because insufficient data are available in adults to judge potential risk in children, and as a Phase 1 trial, there is no known benefit.

Neither women nor minorities will be routinely excluded from participation in the trial. Subjects will be recruited without regard to gender or race. It is expected that race and gender distributions in the trial 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 effects 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 DMID and its 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 data will be released to any unauthorized third party without prior written approval from DMID. 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 subject; or (4) study results which may be published as described in Section 16.

The study monitors or other authorized representatives of DMID may inspect all documents and records required to be maintained by the Site 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 Certificate of Confidentiality

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

The Certificate cannot be used to resist a demand for information from personnel of the United States Government that is used for auditing or evaluation of federally funded projects, like this study, or for information that must be released in order to meet the requirements of the Federal Food and Drug Administration (FDA).

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

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

The release of individual private information or specimens for other research will only occur if consent was obtained from the individual to whom the information, document, or biospecimen pertains, or for the purposes of other research that is in compliance with applicable Federal regulations governing the protection of human subjects in research.

14.7 Study Discontinuation

DMID has the right to terminate the trial or the CTU'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 will 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 will be consistent with the source documents or the discrepancies will 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 will be reviewed by the clinical team and data entry staff, who will ensure that they are accurate and complete. AEs will 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 CTU under the supervision of the Site PI. During the trial, the investigator will 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 study personnel.

ECG data captured by 12-lead Holter monitors will be transmitted to the ECG Core Lab. QT, QTcF, and other ECG interval measurements and ECG interpretations will be performed at the ECG Core Lab using validated protocols. ECG data management is provided by IQVIA Cardiac Safety Services.

15.3 Types of Data

Data for the trial will include clinical safety assessments, laboratory safety assessments, 12-lead standard ECGs, Holter ECG interval measurements and waveform assessments, and PK parameters.

15.4 Timing/Reports

A final CSR will be prepared after all safety, 12-lead standard ECG, 12-lead Holter ECG, and PK data are available. See Section 9.6.2 for additional reporting requirements.

15.5 Study Records Retention

Study files and ICFs will 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 will be retained for a longer period, however, if required by local regulations. No records will be destroyed without the written consent of DMID, if applicable. It is DMID'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 CTU staff. Corrective actions for protocol deviations are to be developed by the CTU 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 will be promptly reported to DMID via DCC's AdvantageEDC^{SM}.

All protocol deviations, as defined above, will be addressed in subject source documents. A completed copy of the DMID Protocol Deviation Form will be maintained in the regulatory file, as well as in the subject's source document. Protocol deviations will 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 study, the lead Principal Investigator is expected to publish the results of this research in a scientific journal. All investigators funded by the NIH must submit or have submitted for them to the National Library of Medicine's PubMed Central (http://www.ncbi.nlm.nih.gov/pmc/) an electronic version of their final, peer-reviewed manuscripts upon acceptance for publication, to be made publicly available no later than 12 months after the official date of publication. The NIH Public Access Policy ensures the public has access to the published results of NIH funded research. It requires investigators to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication. Further, the policy stipulates that these papers must be accessible to the public on PubMed Central no later than 12 months after publication.

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

As of January 2018, all clinical trials supported by the NIH must be registered on ClinicalTrials.gov, no later than 21 days after the enrollment of the first subject. Results of all clinical trials supported by the NIH, generally, need to be submitted no later than 12 months following the primary completion date. A delay of up to 2 years is available for trials that meet certain criteria and have applied for certification of delayed posting. As part of the result posting, a copy of this protocol (and its amendments) and a copy of the Statistical Analysis Plan will be posted on ClincialTrials.gov. For this trial the responsible party is DMID/NIAID/NIH, which will register the trial and post results.

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

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18 APPENDICES

Appendix A: Schedule of Study Procedures and Evaluations

Table 3: Schedule of Events

Study Visit	Screening[1]	Inpatient Periods 1-4			Outpatient Periods 1-4	Final Visit (after Dose 4)	Unscheduled	Early Termination
		Check-in	Dosing	Follow-up				
Study Day / Assessments	**-21 to -2**	**-1**	**1**	**2**	**3 - 7**	**8 (± 2)**		
Informed consent	X							
Inclusion/Exclusion criteria[2]	X	X	X					
Demographics	X							
Medical history	X							
Medical history update		X						
Prior and ConMeds [3]	X	X	X	X	X	X	X	X
Complete PE[4]	X					X		X
Abbreviated PE[5]		X						
Symptom-directed PE[6]			X	X			X	
Height, weight, BMI[7]	X							
Weight		X				X		X
Vital signs[8]	X	X	X	X		X	X	X
Clinical labs (HEM, CHEM, UA)[9]	X	X		X		X	X	X
Viral serology[10]	X							
Serum pregnancy test[11]	X	X				X		X
Serum FSH level[11]	X							
Urine toxicology	X	X						
Alcohol urine test	X	X						
12-lead standard ECG[12]	X	X	X	X		X	X	X
Continuous Holter ECG[13]			X	X				
Randomization[14]			X					
Study drug dosing			X					

Study Visit	Screening[1]	Inpatient Periods 1-4			Outpatient Periods 1-4	Final Visit (after Dose 4)	Unscheduled	Early Termination
		Check-in	Dosing	Follow-up				
Study Day / Assessments	**-21 to -2**	**-1**	**1**	**2**	**3 - 7**	**8 (± 2)**		
PK samples[15]			X	X				X
Counsel on use of appropriate contraception and avoidance of pregnancy[16]	X			X		X	X	X
Counsel to avoid use of prohibited medications, alcohol, marijuana and illicit drugs	X			X				
Counsel to avoid rigorous physical activity	X	X		X				
AE and SAE review[17]			X	X	X	X	X	X
Admit to CTU		X						
Discharge from CTU				X				
Discharge from trial						X		

1. Screening is completed within 21 days before study drug dosing and may require more than one visit.
2. Inclusion/Exclusion criteria at Screening and on Day -1 and Day 1 before dosing in Dosing Period 1 to determine eligibility for enrollment and randomization. I/E criteria will be confirmed before each subsequent dosing period.
3. Prior Medications include prescription during the period 30 days before first dosing and non-prescription drugs, herbs, vitamins, and nutritional supplements taken during the period 15 days before first dosing. ConMeds include those taken after dosing. (See Section 6.6) for prohibited medications and exclusions).
4. Complete PE (except genital, breast, and rectal exams): at Screening, and Final Visit, or ET.
5. Abbreviated PE: on Day -1 (not performed if the complete PE was performed ≤7 days from this visit) in each inpatient period.
6. Symptom-directed PE: on Day 1 (pre-dose and post-dose) and Day 2 in each dosing period to evaluate new symptoms before dosing and AEs after dosing.
7. BMI is calculated as wt (kg) / ht (m^2).
8. VS (BP, HR, RR, T): at Screening; on Days -1, 1, and 2 in each dosing period; and at Final Visit after Dose 4, or ET. On Day 1 of each dosing period, VS at baseline (within 1 h before dosing) and at 1 h (±10 min), 2 h (±10 min), and 4 h (±10 min) after dosing.
9. Clinical laboratory testing with a 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, and Day 2, or ET. Clinical labs may not be repeated before Dose 1 if screening labs were completed ≤7 days before dosing. Clinical labs may be obtained at an unscheduled visit as indicated for work up of an AE.

10. Viral serology (HIV1/2 test, HBsAg, HCV antibody): at Screening.
11. Serum pregnancy test in all women at Screening and within 24 h before each dosing period, and at the Final Visit, or ET. FSH only at Screening in post-menopausal women.
12. 12-lead standard ECG with 10-sec rhythm strip: at Screening, 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], Day 2 (24 h ±2 h) or ET. At an Unscheduled Visit, a 12-lead standard ECG will be done only if needed for the evaluation of an AE.
13. Continuous Holter ECG recorded from 1 h before dosing to 24 h after dosing in each dosing period. ECGs are extracted (three replicates per timepoint) before dosing (at 45, 30, and 15 min before dosing) and at 0.5 h, 1 h, 2 h, 3 h, 4 h, 6 h, 8 h, 12 h, and 24 h after dosing in each dosing period.
14. Eligible subjects are randomized to a treatment sequence on Day 1 before receiving Dose 1. Randomization confirmed at each dosing period.
15. 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), and 24 h (±2 h) after dosing in each dosing period, or ET if it occurs within 24 h of dosing.
16. Female subjects to use appropriate contraception and avoid pregnancy for 30 days after Final Visit or ET. Male subjects to use appropriate contraception and avoid donating sperm for 90 days after Final Visit or ET.
17. Collect all AEs from the time of dosing in Dosing Period 1 through Final Visit after Dose 4. Follow up AEs and SAEs to resolution or stabilization in the clinical judgment of the study investigator.

Appendix B: Acceptable Ranges of Screening Laboratory and Vital Sign Measurements

Table 4: Acceptable Ranges of Screening Laboratory and Vital Sign Measurements – Hematology and Chemistry

HEMATOLOGY AND CHEMISTRY				
Lab test name	**Reference range**	**Acceptable lower limit**	**Acceptable upper limit**	**Lab unit**
Hemoglobin (Hgb), male	13.2 - 17.1	13.0	18.5	g/dL
Hemoglobin (Hgb), female	11.7 – 15.5	11.3	16.5	g/dL
Hematocrit (Hct), male	38.5 – 50.0	38.5	54	%
Hematocrit (Hct), female	35 - 45	35	49	%
White blood cell count (WBC)	3,800 – 10,800	3,500	11,500	cells/µL
Neutrophil count	1,500 – 7,800	1,200	8,500	cells/µL
Lymphocyte count	850 – 3,900	800	5,000	cells/µL
Monocyte count	200 - 950	0	1,100	cells/µL
Eosinophil count	150 - 500	0	600	cells/µL
Basophil count	0-200	0	300	cells/µL
Platelet count	140 - 400	125	475	10^3/µL
Sodium	135 - 146	132	148	mmol/L
Potassium	3.5 - 5.3	3.3	5.5	mmol/L
CO_2 (Bicarbonate)	20 - 32	19	33	mmol/L
Chloride	98 -110	94	115	mmol/L
Calcium	8.6 - 10.3	8.0	11.0	mg/dL
Magnesium	1.5 - 2.5	1.1	2.8	mg/dL
Blood urea nitrogen (BUN)	7 – 25	0	28	mg/dL
Glucose, fasting	65 - 99	50	115	mg/dL
Serum creatinine, male	0.6 – 1.35	0.5	1.45	mg/dL
Serum creatinine, female	0.5 – 1.10	0.4	1.2	mg/dL
Direct bilirubin	0.0-0.2	0	0.4	mg/dL
Total bilirubin	0.2 – 1.2	0	1.4	mg/dL
Total protein	6.1 - 8.1	5.8	8.6	g/dL
Albumin	3.6 – 5.1	3.2	6.0	g/dL
Aspartate transferase (AST), male	10 - 40	0	45	U/L
Aspartate transferase (AST), female	10 - 30	0	35	U/L
Alanine transferase (ALT), male	9 - 46	0	52	U/L
Alanine transferase (ALT), female	5 - 32	0	38	U/L
Alkaline phosphatase, males	40 - 115	0	150	U/L
Alkaline phosphatase ,females	33 – 115	0	150	U/L
Hepatitis B surface antigen (HBsAg)	non-reactive	non-reactive	non-reactive	n/a
Hepatitis C antibody (HCVAb)	non-reactive	non-reactive	non-reactive	n/as
HIV-1/ HIV-2 antibody	non-reactive	non-reactive	non-reactive	n/a

Serum β-HCG (females only)	negative	negative	negative	n/a
FSH (post-menopausal females only)	23 – 116.3	23	116.3	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. These 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 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 will be provided or the 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 values that reach toxicity Grade 1 values are allowable.
f. Serum chloride and albumin values above the ULN are 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]

Table 5: Acceptable Ranges of Screening Laboratory and Vital Sign Measurements – Urinalysis

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)	0-2	0	6	/field
Bacteria	none	few	[b]	/field
Hyaline casts	none	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

Table 6: Acceptable Ranges of Screening Laboratory and Vital Sign Measurements – Vital Signs

Measurement	Lower limit	Upper limit	Lab unit
Systolic BP	90	150	mmHg
Diastolic BP	40	90	mmHg
Pulse rate	45	100	bpm
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 CTU will 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.

Table 7: Toxicity Grading Tables – Clinical AEs

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 • Baseline >60 OR, if • Baseline <60	• 50-54 OR, if Baseline <60, • 45-50	• 45-49 OR, if baseline <60, • 40-44	• <45 OR, if baseline <60 • <40	As above
Hypertension (systolic) - mmHg	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) - mmHg	91-95	96-100	>100	As above
Hypotension (systolic) - mmHg	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 VS would not be considered toward halting criteria. Abnormalities of VS will be described as "increased X" or "decreased X" (X = HR, BP, RR, 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 will 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 VS that are either secondary to systemic or organ-specific clinical syndrome or primary disorders will be coded in the appropriate SOC (e.g., "cardiac disorders", respiratory disorders", "immunological disorders", etc.). These abnormalities will 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	
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	

Clinical AEs	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Comments
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 OR angioedema OR anaphylaxis OR requires epinephrine	
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)	

Clinical AEs	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Comments
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)	Require minimal or no treatment; does not interfere with the subject's daily activities.	Result in a low level of inconvenience or concern with therapeutic measures; may cause some interference with functioning.	Interrupt a subject's usual daily activity and may require systemic drug therapy or other treatment; are usually incapacitating	

Table 8: Toxicity Grading Tables – Laboratory AEs

Laboratory AEs	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Blood, serum, or plasma *			
Sodium decrease – mmol/L	130 - 131	125 –129	<125
Sodium increase – mmol/L	149 - 150	151 – 153	>153
Potassium increase – mmol/L	5.6 – 6.0	6.1 – 6.4	≥6.5
Potassium decrease – mmol/L	3.0 – 3.2	2.5 – 2.9	<2.5
Bicarbonate (CO_2) increase – mmol/L	34 - 35	36 - 37	>37
Bicarbonate (CO_2) decrease – mmol/L	17 - 18	14 - 16	<14
Glucose decrease, fasting – mg/dL	47 - 49	45 –46	<45
Glucose increase, fasting – mg/dL	116 – 125	126 – 249	≥250
Glucose increase, non-fasting – mg/dL	116 – 160	161 – 249	≥250
Blood urea nitrogen – mg/dL	25 - 27	28 – 32	>32
Creatinine increase, male – mg/dL	1.46 – 1.7	1.71 – 2.3	>2.3
Creatinine increase, female – mg/dL	1.21 – 1.5	1.51 – 2.0	>2.0
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
Total protein decrease – g/dL	5.1 – 5.7	4.6 – 5.0	<4.6
Albumin decrease – g/dL	2.8 – 3.4	2.5 – 2.7	<2.5
AST increase, male – U/L	46 – 115	116 – 220	>220
AST increase, female – U/L	36 - 90	91 - 175	>175
ALT increase, male – U/L	53 – 115	116 - 230	>230
ALT increase, female – U/L	39 – 95	96 – 190	>190
Alkaline phosphatase increase – U/L	151 – 240	241 – 360	>360
Total bilirubin (serum) increase – mg/dL (with other LFTs in the normal range)	1.5 – 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)**	1.5 – 1.7	1.8 – 2.1	>2.1
Hemoglobin decrease, female – g/dL	10.8 – 11.2	9.4 – 10.7	<9.4
Hemoglobin decrease, male – g/dL	11.8 – 12.9	10.0 – 11.7	<10.0
WBC increase – cells/µL	11,000 – 15,000	15,001 – 20,000	>20,000
WBC decrease – cells/µL	2,500 – 3,500	1,500 – 2,499	<1,500
Neutrophils decrease – cells/µL	1,200 – 1,499	1,000 – 1,199	<1,000
Lymphocytes decrease – cells/µL	750 – 849	500 – 749	<500

Laboratory AEs	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Monocytes increase – cells/µL	1,101 – 2,000	2,001 – 3000	>3,000
Eosinophils increase – cells/µL	500 – 750	751 – 1,500	>1,500
Basophils increase – cells/µL	201 – 500	501 – 800	>800
Platelets decrease – cells/µL	120,000 – 124,000	100,000 – 119,000	<100,000
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	6-10	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 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 CTU clinician and reported as Grade 1 abnormalities 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 CTU clinician.
Note 3: Other laboratory parameters performed and reported as parted of the CBC, metabolic panel, and UA will be evaluated by the study clinician, recorded in the source document, reported as laboratory AEs if clinically significant, and graded according to the criteria in Section 9.2.1.
Note 4: If the dipstick UA 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 dipstick UA or microscopic UA may be retested following cessation of menses.

Table 9: Toxicity Grading Tables – ECG

ECG interval abnormality	Grade 1	Grade 2	Grade 3
QTcF interval prolonged (msec)	Asymptomatic, QTcF 450-479 msec	Asymptomatic, QTcF 480-500 msec OR increase in interval 30-59 msec above baseline	Asymptomatic, QTcF >500 msec OR increase in interval ≥60 msec above baseline
PR interval prolonged (msec)	210-250 msec	>250 msec	Type II 2nd degree AV block OR ventricular pause >3.0 sec

Note: Events will be coded as SAEs if there are life-threatening associated symptoms or signs (arrhythmia, CHF, hypotension, syncope, TdP, etc.)
Note: If a male subject was accepted into the trial with a QTcF value that overlaps with values used for grading Grade 1 QTcF prolongation, an AE will be reported if the QTcF value is higher than the baseline value

Appendix D: Blood Volume Withdrawn During the Trial

Table 10: Laboratory Samples and Estimated Total Blood Volume (mL)

Study Periods		**Inpatient Periods 1 to 4**			**Outpatient Periods 1 - 4**	**Dosing Period 4**	
Study Visit	**Screen**	**Check-in**	**Dosing**	**Follow-up**	**Wash-out**	**Final**	**ET**
+Study Day[a]	**-21 to -2**	**-1**	**1**	**2**	**3-7**	**8 (±2)**	
+							
HEMATOLOGY[1]	4	4		4		4	4
CHEMISTRY and serum β-HCG and FSH[1]	5	5		5		5	5
Viral Serology (HIV, HBsAg, HCV)[1]	8.5						
PK[3]			54	6			6
Total volume/visit	17.5	9	54	15	0	9	15
Dosing periods multiplier	1	4	4	4		1	1
Total volume (all dosing periods)	17.5	36	216	60	0	9	15
Cumulative total volume	17.5	53.5	269.5	329.5	329.5	338.5	

[a] Study Days shown correspond to days in each treatment period. For a view of the cumulative numbering of study days, please refer to Section 7.

[1] Clinical blood tests (HEM, CHEM) are drawn at Screening Visit, on Day -1, and on Day 2 in each treatment period, or ET. Serum pregnancy test in all women at Screening and within 24 h before each dosing period, and at the Final Visit, or ET. FSH only at Screening in post-menopausal women.

[2] Viral serology tests are drawn at Screening.

[3] PK serum samples are drawn on Day 1: 30 min before dosing and at 0.5, 1, 2, 3, 4, 6, 8, 12, and 24 h after dosing in each dosing period, or ET if occurs within 24 h of dosing.

Appendix E: A Partial List of Drugs Causing QTc Prolongation and Torsade de Pointes

Table 11: A Partial List of Drugs Causing QTc Prolongation and Torsade de Pointes

CATEGORY	DRUG
Anti-arrhythmic agents	Amiodarone Disopyramide Dofetilide Dronedarone Flecainide Ibutilide Procainamide Quinidine Sotalol
Anti-infective agents	Azithromycin Ciprofloxacin Clarithromycin Erythromycin Levofloxacin Moxifloxacin
Anti-malarial agents	Chloroquine
Anti-psychotic agents	Chlorpromazine Droperidol Haloperidol Pimozide Thioridazine
Anesthetics, General	Propofol Sevoflurane
Others	Methadone Papaverine HCl

Source: https://crediblemeds.org/ (accessed 19 December 2017) and Reference 17