A Phase 1 Safety and Intrapulmonary Pharmacokinetics Study of ZTI-01 (Intravenous Fosfomycin Disodium) in Healthy Adult Subjects

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STATEMENT OF COMPLIANCE

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

- United States (US) 45 Code of Federal Regulations (CFR) Part 46: Protection of Human Subjects
- Food and Drug Administration (FDA) Regulations, as applicable: 21 CFR Part 50 (Protection of Human Subjects), 21 CFR Part 54 (Financial Disclosure by Clinical Investigators), 21 CFR Part 56 (Institutional Review Boards), and 21 CFR Part 11, 21 CFR Part 312 (Investigational New Drug Application).
- International Conference on Harmonization (ICH) E6:GCP; 62 Federal Register 25691 (1997); and future revisions
- Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research, Report of the National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research
- National Institutes of Health (NIH) Office of Extramural Research, Research Involving Human Subjects, as applicable
- National Institute of Allergy and Infectious Diseases (NIAID) Clinical Terms of Award, as applicable
- Applicable Federal, State, and Local Regulations and Guidance

SIGNATURE PAGE

The signature below provides the necessary assurance 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 E6: GCP guidelines.

I agree to conduct the study in compliance with GCP and applicable regulatory requirements.

I agree to conduct the study in accordance with the current protocol and will not make changes to the protocol without obtaining the Sponsor's approval and Institutional Review Board (IRB)/Independent Ethics Committee (IEC) approval, except when necessary to protect the safety, rights, or welfare of subjects.

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LIST OF ABBREVIATIONS

AE	Adverse Event/Adverse Experience
ALT	Alanine Aminotransferase
AM	Alveolar Macrophage
ALP	Alkaline Phosphatase
AST	Aspartate Aminotransferase
AUC	Area under the concentration-time curve
AUC_{0-t}	AUC calculated to the last measured concentration
AUC_{0-inf}	AUC extrapolated to infinity
BAL	Bronchoalveolar lavage
BMI	Body Mass Index
C_{max}	Maximum Measured Concentration
CRF	Case Report Form
CDC	Centers for Disease Control and Prevention
CFR	Code of Federal Regulations
CFU	Colony Forming Unit
CL	Clearance
CrCl	Creatinine Clearance
CRE	Carbapenem-Resistant Enterobacteriaceae
CROMS	Clinical Research Operations and Management Support
DCC	Data Coordinating Center
DEPRU	Duke Early Phase Research Unit
DHHS	Department of Health and Human Services
DMID	Division of Microbiology and Infectious Diseases

DMID-CMS	DMID Clinical Materials Services (CMS, Fisher BioServices)
ECG	Electrocardiograph
ELF	Epithelial Lining Fluid
ESBL	Extended-Spectrum Beta-Lactamase
FDA	Food and Drug Administration
FSH	Follicle-Stimulating Hormone
FWA	Federal Wide Assurance
GCP	Good Clinical Practice
GI	Gastrointestinal
HBsAg	Hepatitis B Surface Antigen
HCV	Hepatitis C Virus
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonization
ICMJE	International Committee of Medical Journal Editors
IM	Intramuscular
IND	Investigational New Drug Application
IRB	Institutional Review Board
ISM	Independent Safety Monitor
IV	Intravenous
LC-MS/MS	Liquid chromatography-mass spectrometry/mass spectrometry
MIC	Minimum Inhibitory Concentration
MOP	Manual of Procedures
MDR	Multi-Drug Resistant
MRSA	Methicillin Resistant *S. aureus*

N	Number (typically refers to subjects)
NCI	National Cancer Institute
NDA	New Drug Application
NEJM	New England Journal of Medicine
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
OCRA	Office of Clinical Research Affairs
OHRP	Office for Human Research Protections
OHSR	Office for Human Subjects Research
ORA	Office of Regulatory Affairs
PD	Pharmacodynamics
PHI	Protected Health Information
PI	Principal Investigator
PIP-TAZ	Piperacillin-Tazobactam
PK	Pharmacokinetics
PRSP	Penicillin Resistant *S. pneumonia*
PST	Product Support Team
QA	Quality Assurance
QC	Quality Control
RIC	Resistance Inhibitory Concentration
RLD	Reference Listed Drug
SAE	Serious Adverse Event/Serious Adverse Experience
SMC	Safety Monitoring Committee
SOP	Standard Operating Procedure
$T_{½}$	Terminal Elimination Half-Life

T_{max}	Time to Maximum Concentration
TEAE	Treatment-Emergent Adverse Event
TOC	Test-of-Cure Visit
US	United States
USP	United States Pharmacopeia
UTI	Urinary Tract Infections
V_d	Volume of Distribution
VRE	Vancomycin Resistant Enterococci
WFI	Sterile Water for Injection

PROTOCOL SUMMARY

Title:	A Phase 1 Safety and Intrapulmonary Pharmacokinetics Study of ZTI-01 (Intravenous Fosfomycin Disodium) in Healthy Adult Subjects
Phase:	1
Population:	40 healthy male and female adults will be enrolled and randomized (to obtain 30 evaluable subjects) aged 18-45 years, inclusive, with BMI 18-30 kg/m^2, inclusive, and body weight >50 kg (110 lbs)
Number of Sites:	1 (Duke Early Phase Research Unit [DEPRU], Durham, NC)
Study Duration:	Approximately 6 months
Subject Participation Duration:	Individual subject participation will be approximately 33 days to include up to 30 days between screening and baseline/enrollment, 2 days treatment (dosing) and 1 day of post-dosing follow-up
Description of Agent or Intervention:	ZTI-01 [intravenous (IV) fosfomycin disodium] packed in a single Type 1 glass vial equivalent to 4 g of fosfomycin and 1.28 g of sodium with inactive ingredient of recrystallized succinic acid.
Objectives:	<u>Safety:</u> • Determine the safety of multiple doses of ZTI-01 (6 g dose administered as a 1-hour IV infusion (+10 minute window), every 8 hours, for a total of 18 g in three doses) <u>Pharmacokinetic:</u> • Determine the plasma and intrapulmonary pharmacokinetics (PK) (epithelial lining fluid, and alveolar macrophage concentrations of fosfomycin) after multiple doses of ZTI-01 (6 g dose administered

as a 1-hour IV infusion (+10 minute window), every 8 hours, for a total of 18 g in three doses)

Outcome Measures:

Safety:

- The occurrence of adverse events (AEs), summary of physical exam findings, vital signs, clinical laboratory tests, and ECGs (B) at any time from the start of study drug administration through the end of subject follow-up

Pharmacokinetic:

- Plasma ZTI-01 concentrations will be measured by a validated LC-MS/MS assay for the blood samples collected during the study (Table 3). PK parameters of ZTI-01 will be estimated from the plasma concentration including maximum measured plasma concentration (C_{max}), area under the concentration vs. time curve (AUC_{0-8}), time to peak concentration (t_{max}), terminal elimination half-life ($t_{1/2}$), terminal-phase elimination rate constant (λ_z), volume of distribution (Vd), and clearance (CL).

- Intrapulmonary PK of ZTI-01 defined as percent penetration of lung epithelial lining fluid (ELF) and alveolar macrophages (AMs). The ratios of ELF and AM concentrations of fosfomycin to simultaneous plasma concentrations will be calculated for each subject and summarized for each sampling time. The median concentrations of fosfomycin from the BAL sampling times will be used to estimate the AUC_{0-8} of plasma, ELF, and AM. The ratio of AUC_{0-8} of ELF-to-plasma and AM-to-plasma will be calculated to determine the percent penetration.

Description of Study Design:

This is a Phase 1, open-label, multiple-dose study conducted at a single center. The treatment period will consist of 18 g of ZTI-01 in three divided doses (Table 1). A total of 30 enrolled subjects will be randomized to undergo a single standardized bronchoscopy with bronchoalveolar lavage (BAL) at one of five sampling times as defined below (Table 2). A total of 6 subjects will be assigned to each BAL-

	sampling time. Ten additional enrolled subjects will act as alternates to obtain 30 evaluable subjects. Each subject will complete Screening, Baseline, Treatment and Follow-up Phases. The Screening Phase will be conducted on an outpatient basis within 30 days of the Baseline Phase, which will consist of admission to DEPRU on the day before the first dose (Day -1) for pre-dosing assessments. The Treatment Phase will start on the day of first dose (Day 1) and end at the time of discharge from DEPRU. Three IV doses of ZTI-01 will be administered every 8 hours (hours 0, 8, and 16 during the Treatment Phase). Blood samples for PK measurements will be collected before each dose, up to 8 hours after the start of the first IV infusion of ZTI-01, and up to 12 hours after the start of the third IV infusion (Tables 2 and 3). One bronchoscopy with BAL will be completed on each subject at the timepoint to which that subject was randomized. Key electrolytes will be remeasured prior to each subject's bronchoscopy in order to minimize risk of arrhythmia. Subjects will be discharged following the final 12-hour blood sampling time point (if safety parameters are acceptable to the Investigator). Safety assessments will be conducted during the entire 24-hour Treatment Phase. The Follow-Up Phase consists of a telephone interview on Day 3 for symptom-driven safety assessments.
Estimated Time to Complete Enrollment:	Approximately 12 weeks

Table 1: Study drug administration

Study drug	Formulation/Strength	Each dose/Route	Dosing interval	Total no. of doses
ZTI-01	Fosfomycin, disodium salt, 4 g fosfomycin per vial	6 g fosfomycin per 200 mL dose, 1-hour IV infusion (+10 minute window)	Every 8 hours	3

Table 2: Bronchoscopy with BAL sampling times

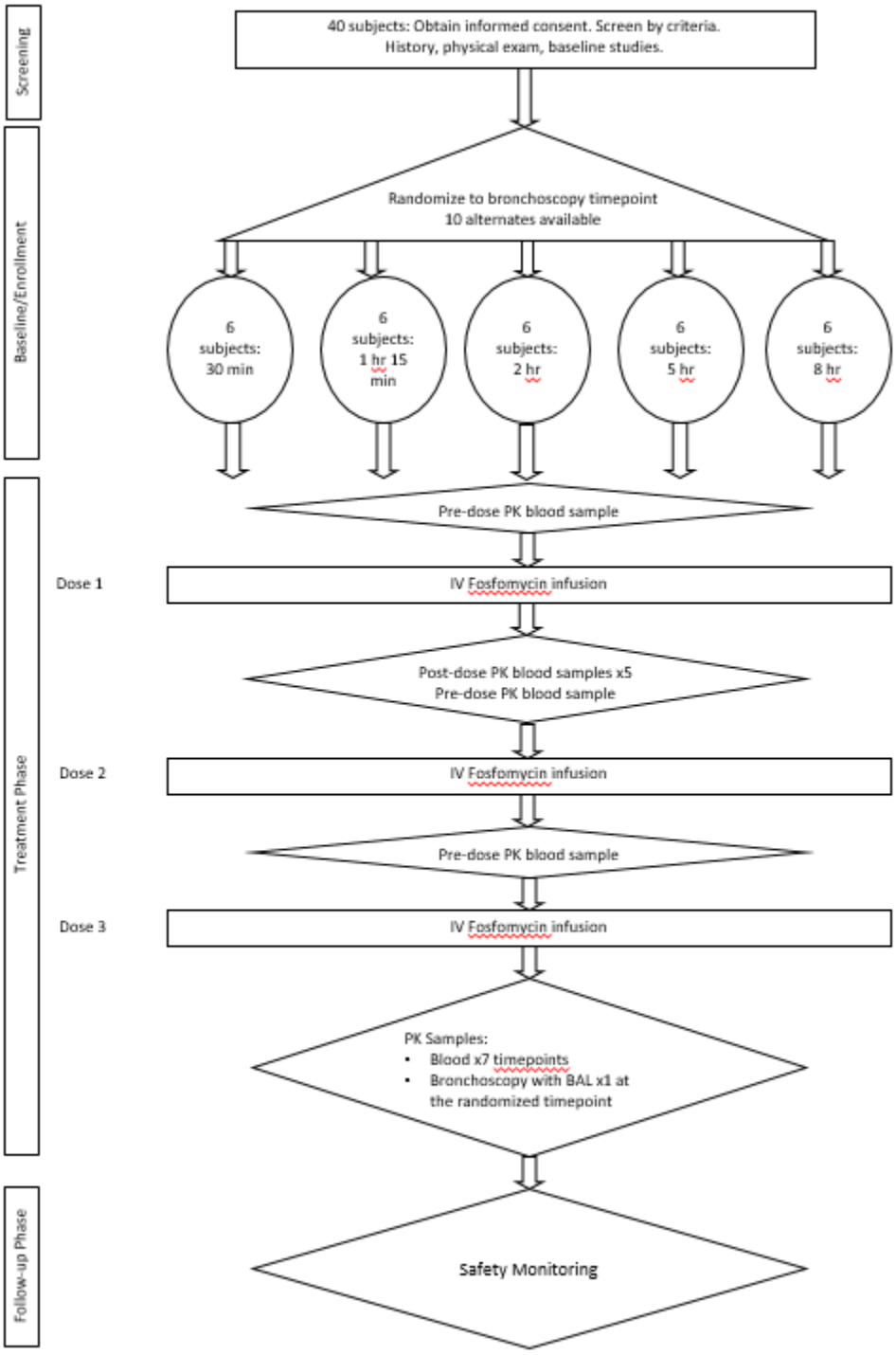
	BAL sampling times after start of third IV infusion of ZTI-01*				
	30 minutes	1 hour 15 minutes	2 hours	5 hours	8 hours
No. subjects (n=30 completed)	6	6	6	6	6

*Each subject will complete a single standardized bronchoscopy with BAL randomized to one of the five sampling times shown.

Table 3: Blood PK sampling times

Dosing	Dose 1		Dose 2	Dose 3	
	Pre-Dose	During/After Dose	Pre-Dose	Pre-Dose	During/After Dose
Timing of PK blood sample	Within 10 minutes before starting the 1-hour infusion	30 minutes, 1 hour, 1 hour 15 minutes, 2, and 5 hours after starting the first dose	Within 10 minutes before starting the 1-hour infusion	Within 10 minutes before starting the 1-hour infusion	30 minutes, 1 hour, 1 hour 15 minutes, 2, 5, 8, and 12 hours after starting the third (last) dose

Figure 1: Schematic of Study Design



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2. BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1. Background Information

Bacterial resistance against antimicrobials has reached alarming levels in recent decades resulting in crisis and necessitating the need for more antibiotic treatment options, particularly among Gram-negative bacteria, including those that express extended-spectrum beta-lactamases (ESBLs) and the carbapenem-resistant Enterobacteriaceae (CRE). The lack of available and effective antibiotics for these organisms has created an unmet medical need that is widely acknowledged (Alemayehu et al, 2012). Infections with CRE are difficult to treat and there are limited treatment choices available or options suitable for certain patients. Mortality rates as high as 40%-50% have been associated with CRE infection, and thus the Centers for Disease Control and Prevention (CDC) considers this an urgent threat to public health (CDC Report 2013).

Since the first report in a US hospital in 2001, CRE prevalence in the US has increased rapidly with an estimated 6% of all hospitals having reported at least one such infection. Data from the European Antimicrobial Resistance Surveillance Network (EARS-Net) showed statistically significant increases in the prevalence of carbapenem resistance among *K. pneumoniae* isolates from 2005 to 2010 in progressively more countries across Europe (Magiorakos et al, 2013). Given the rapid rise of carbapenem resistance outside the US, it is very probable that we will see increasing rates in the US as well. In particular, rising rates among long-term-care facilities, where an estimated 25% of elderly persons in the US will reside at some point in their lives, is alarming. In a cross-sectional study of facility residents seen in the emergency department for urinary tract infections (UTIs), multidrug-resistant (MDR) bacteria were identified in up to 80% of isolates (Genao and Buhr, 2012).

There has been a renewed interest and effort to expedite the availability of antibiotics effective against these resistant pathogens. To fill the void of desperately needed therapeutic options, a re-evaluation of "forgotten," older antibiotics and their different formulations has been proposed (Kanj and Khanafani, 2011; Pulcini et al, 2012). Despite years of safe and effective use, many of these drugs require a "modernization" of PK-pharmacodynamics (PD) to adequately bridge clinical data and thereby ensure successful therapeutic results in the era of increased resistance. Evidence suggests that the development of resistance is associated with the use of suboptimal dosing regimens, while optimal dosing regimens are associated with a higher likelihood of suppressing the development of resistance (Drusano, 2003). For this to be feasible, alternative approaches to the development of these forgotten antibiotics must be employed and used in support of marketing approval.

ZTI-01 is fosfomycin for injection, a phosphonic acid derivative formulated as a disodium salt. Fosfomycin acts by inhibiting peptidoglycan assembly, thereby disrupting cell wall synthesis (Kahan et al., 1974). Uptake into the bacterial cell occurs via active transport, by the L-α-glycerophosphate transport

and hexose phosphate uptake systems. Once inside the bacterium, fosfomycin competes with phosphoenolpyruvate to irreversibly inhibit the enzyme enolpyruvyl transferase, which catalyzes the first step of peptidoglycan synthesis, and thereby interrupt cell wall synthesis.

The antimicrobial spectrum of fosfomycin is very broad. It exhibits bactericidal activity against anaerobic pathogens and many Gram-positive and -negative bacteria, including the increasingly problematic CRE and ESBL-producing organisms. *Staphylococcus aureus* (including MRSA), *Streptococcus pneumoniae* (including PRSP), *Escherichia coli*, *Neisseria meningitidis*, *Haemophilus influenzae*, *Klebsiella pneumoniae*, *Enterococcus faecalis* (including VRE), *Serratia marcescens*, *Pseudomonas aeruginosa*, and *Proteus mirabilis* are among the many organisms in which fosfomycin is active (Michalopoulos et al, 2011).

In Europe and Japan, IV fosfomycin has provided a safe and effective option for treating patients with UTIs and a variety of other, often very severe, infections, including MDR infections in patients with cystic fibrosis (Mirakhur et al, 2003) and sepsis (Matzi, 2010) for more than 45 years. ZTI-01 penetrates rapidly into tissues and achieves clinically relevant concentrations in serum, urine, soft tissues, lung, bone, cerebrospinal fluid, and heart valves, making it a desirable antibiotic treatment option (Roussous et al, 2009). Falagas et al (2008) compiled literature evidence regarding the effectiveness and safety of oral, IV, and intramuscular (IM) fosfomycin for the treatment of patients with Gram-positive and/or Gram-negative bacterial infections, excluding UTI and gastrointestinal (GI) infection. Of the 1311 potentially relevant studies, 62 studies involving 1604 patients with a variety of infections (pneumonia and other respiratory infections; osteomyelitis; meningitis; ear, nose, and throat infections; surgical infections; obstetric and gynecological infections; arthritis; septicemia; peritonitis; cervical lymphadenitis; eye infections; diabetic foot infections; and typhoid fever) were included. The most frequently involved pathogens were *S. aureus* and *P. aeruginosa*, and fosfomycin was administered via oral, IV, or IM routes at dosages of 2-24 g daily, administered in divided doses every 6-8 hours. The mean duration of fosfomycin treatment ranged from 5 to 21 days, including fosfomycin administered alone or in combination with other antibiotics. Cures were achieved in 81.2% (1242/1529) of patients, and clinical improvement was noted in 3.0% (46/1529) of patients.

Among 664 patients in these studies, fosfomycin treatment was also well tolerated: 5.4% reported mild GI symptoms (e.g., nausea, diarrhea, epigastralgia, vomiting); 4.0% developed skin manifestations (e.g., rash, dermatitis, exanthema); 3.0% experienced pain at the injection site or phlebitis; and 2.1%, 1.2%, and 0.1% showed moderate increases in platelet count, eosinophil count, and transaminase level, respectively.

In general, fosfomycin shows little toxicity, and adverse effects are generally mild following parenteral doses up to 24 g/day. Renal impairment significantly decreases the excretion of fosfomycin and doses should be reduced if the creatinine clearance is less than 50 ml/min. Since fosfomycin shows synergistic action with other antimicrobials, it is often used in combination (Kastoris et al, 2010). In fact, treatment with fosfomycin is considered free from the nephrotoxicity that characterizes treatment with aminoglycosides. Animal studies have demonstrated that fosfomycin protects against nephrotoxicity by inhibiting aminoglycoside-induced histamine release from mast-cell destruction. Fosfomycin also

increases oxygen levels in mitochondria and cyclic-AMP levels in mast cells (Walwarawooth, 2004; Popovic et al, 2010).

Despite decades of use, fosfomycin has retained excellent antimicrobial activity. The emergence of MDR organisms such as fluoroquinolone-resistant *E. coli*, ESBL-producing bacteria, and carbapenem-resistant *K. pneumoniae* has prompted the re-evaluation of fosfomycin, due to the dwindling number of effective antimicrobial options (Pulcini et al, 2012).

ZTI-01 has been granted Qualified Infectious Disease Product (QIDP) and Fast Track designations by the FDA as an antibiotic drug intended to treat serious or life-threatening infections, including those caused by MDR or emerging pathogens, for which limited or no antibacterial therapy has been developed. Two clinical studies (DMID-13-0064 and ZEUS) support US registration of ZTI-01.

A Phase 1, single-dose, randomized, three-period, crossover study (Wenzler, 2017) was conducted at a single center to assess the safety, tolerability, and PK of ZTI-01 (fosfomycin for injection) in healthy adults. Subjects were randomized to receive one of three treatment sequences (1 or 8 g ZTI-01 administered as a 1-hour IV infusion (+10 minute window) or 3 g fosfomycin tromethamine [sachet, Monurol®] administered orally per label instructions). Each treatment sequence was enrolled in parallel, and each subject received all three treatments in crossover fashion. Each crossover period included a single dose of study drug under fasted conditions, followed by safety assessments and blood/urine collections for PK measures up to 48 hours. Each treatment period was separated by a 3- to 7-day washout period.

A total of 30 healthy subjects were enrolled and randomized, and 28 of them completed all study procedures. The mean age was 27 years (range, 18-44). There were no serious adverse events (SAEs), and no subjects discontinued treatment due to treatment-emergent adverse events (TEAEs).

The incidence of TEAEs was 39.3% in the ZTI-01 IV (1-g) group, 67.9% in the ZTI-01 IV (8-g) group, and 43.3% in the oral Monurol group. The most common TEAEs (>2 subjects in any group) in the ZTI-01 IV (1-g), ZTI-01 IV (8-g), and Monurol groups were decreased heart rate (10.7, 28.3, and 16,7%), decreased blood calcium (17.9, 10.7, and 13.3%), and headache (10.7, 7.1, and 3.3%), respectively. None of the reported TEAEs was considered clinically significant based on clinical evaluation and course. Abnormal laboratory findings were mild, asymptomatic, and not considered clinically significant. The most common (affecting >2 subjects) laboratory abnormality was hypocalcemia. There were no clinically significant changes in vital signs in any subject during the study. The most common (affecting >2 subjects) abnormality in vital sign measurements was a decreased heart rate. A decrease in systolic blood pressure was reported by 1 subject in the ZTI-01 IV (1-g) group, considered mild in severity and related to study treatment by the Investigator. There were no subjects with abnormal ECG intervals.

A Phase 2/3, multicenter, randomized, double-blind, comparative study (ZEUS, NCT02753946, unpublished) to evaluate the safety and efficacy of ZTI-01 (fosfomycin disodium) vs. piperacillin/ tazobactam (PIP-TAZ) in the treatment of complicated UTIs, including acute pyelonephritis (AP), in

hospitalized adults was recently completed. A total of 465 subjects were randomized at 72 sites in Central and Eastern Europe and the US. The mean age of treated patients was 50 years (range, 18-89) across treatment arms, and most (63%) were female. About 30% of patients had sepsis at baseline, based on systemic inflammatory response syndrome (SIRS) criteria, and 9% of patients had bacteremia at baseline.

Subjects were randomized (1:1) to receive a fixed duration of 7 to 14 days of either ZTI-01 (6 g) IV infused over 1 hour every 8 hours (18 g/day), or PIP-TAZ (4 g piperacillin/0.5 g tazobactam) IV infused over 1 hour every 8 hours. All but 1 subject (in the PIP-TAZ group) received at least 1 dose of study drug. Most subjects completed study drug treatment: 219 (94.0%) subjects in the ZTI-01 group and 222 (95.7%) subjects in the PIP-TAZ group.

The primary efficacy endpoint was the proportion of subjects with an overall success (clinical cure and microbiologic eradication) in the m-MITT Population at the test-of-cure (TOC) Visit. Overall responses were programmatically determined, and microbiological response presumed that the same bacterial species were identical at the Screening and TOC Visits. Overall success was higher in the ZTI-01 group (64.7%) compared to the PIP-TAZ group (54.5%). The treatment difference was 10.2% (95% CI, -0.4 to 20.8) and the lower bound of the 95% CI met the prespecified non-inferiority margin of -15%, demonstrating that ZTI-01 was non-inferior to PIP-TAZ.

The microbiologic eradication analysis (part of Overall Success determination) presumed that any pathogen strains recovered at baseline and again at TOC were identical. Patient isolates from both treatment arms underwent blinded pulse-field gel electrophoresis analysis. These molecular data confirmed 20 strains with unique identity and a reanalysis of the primary objective was performed. Using this classification, higher overall success rates for both treatment arms was observed; ZTI-01 (69.0%) compared to PIP-TAZ (57.3%); the treatment difference was 11.7% (95% CI, 1.3 to 22.1), indicating that ZTI-01 was superior to PIP-TAZ.

Both study drugs were well-tolerated, and study drug discontinuations or interruptions due to AEs were uncommon. The incidence of TEAEs was higher in the ZTI-01 group (42.1%) compared to the PIP-TAZ group (32.0%); however, most TEAEs in the ZTI-01 group were well-described class effects, including asymptomatic laboratory abnormalities (e.g., elevated liver transaminases, hypokalemia) or GI disorders. The incidence of SAEs was low and similar in the ZTI-01 (5 [2.1%]) and PIP-TAZ (6 [2.6%]) groups. No unique SAE occurred in >1 subject in either treatment group. There were no deaths and only one SAE deemed related to ZTI-01 (hypokalemia) in the study.

Table 4: AEs occurring in ≥2% of patients receiving ZTI-01 in a phase 2/3 clinical trial

Adverse event (%)	Complicated UTIs, including pyelonephritis	
	ZTI-01 (N=233)	Piperacillin-Tazobactam (N=231)
Nausea	4.3	1.3
Diarrhea	3.9	4.8
Vomiting	3.9	0.4
Phlebitis	0.9	2.6
Increased ALT	8.6	2.6
Increased AST	7.3	2.6
Hypokalemia	6.4	1.3
Headache	2.6	2.2

2.2. Rationale

Given the urgent medical need and the spread of bacterial resistance, ZTI-01 may provide a useful option for health care providers. ZTI-01 is a proven and useful therapeutic agent with more than 45 years of clinical experience documenting that both oral and IV formulations are effective and well-tolerated in a variety of patient populations. In addition to the FDA-approved oral formulation, an IV formulation of fosfomycin (ZTI-01, fosfomycin for injection) would represent a useful therapy option for difficult-to-treat infections and thus an important addition to the US therapeutic armamentarium.

Intrapulmonary penetration of antibiotics is considered a crucial factor in their ability to treat lower respiratory tract infections (Rodvold et al, 2011; Ambrose et al, 2010). Pulmonary epithelial lining fluid (ELF) and alveolar macrophages (AMs) have been described as important sites of infection with common extracellular and intracellular respiratory pathogens, respectively. Measurement of drug concentrations in ELF and AMs by bronchoscopy with BAL has become a practical and reliable method to assess intrapulmonary penetration. Knowledge of a drug's penetration (i.e., time course of drug entry and magnitude of drug concentration) in different lung compartments, and the most-likely pathogen's location within the lung, can assist in the design of antibiotic dosing regimens to effectively treat lower respiratory tract infections.

The development pathway for ZTI-01 includes evaluating treatment of patients with serious lower respiratory tract infections. It is anticipated that this trial will provide sufficient evidence on two development fronts. The first of these is to determine the concentrations of fosfomycin in the ELF and AMs relative to fosfomycin concentrations in plasma. The second is to provide adequate information to establish safety of the selected dosing regimen to enable further evaluation of ZTI-01 in the intended patient population.

This trial will evaluate a single dose sequence of ZTI-01: three 6-g doses of fosfomycin, administered as a 1-hour IV infusion (+10 minute window), every 8 hours. The dosage selection was directly influenced by applying PK-PD principles of anti-infective agents. Recent studies have provided useful information for

modernizing and optimizing fosfomycin dosing regimens. VanScoy et al found that the PK-PD parameter that most strongly associated with efficacy was the percentage of fosfomycin concentrations above the resistance inhibitory concentration (RIC) [% T>RIC] during the dosing interval (VanScoy et al, 2015). The proposed magnitude % T>RIC associated with net bacterial stasis and a 1-$\log_{10}$ and 2-$\log_{10}$ colony forming unit (CFU) reduction in bacterial density over 24 hours was 11.8, 20.9, and 32.7%, respectively. Total daily fosfomycin doses of 8 g and 32 g resulted in a rapid reduction in bacterial density without amplification of a drug-resistant subpopulation. A subsequent study suggested that the lowest fosfomycin dosing regimen that did not amplify a drug-resistant bacterial subpopulation was 4 g administered every 8 hours (VanScoy, et al 2016). An in-vivo study performed in a neutropenic murine thigh model (Lepak et al, 2015) used dose fractionation to assess PK-PD targets and survival. This study demonstrated ZTI-01 potency against *E. coli*, *K. pneumoniae*, and *P. aeruginosa*, including ESBL-producing and carbapenem-resistant phenotypes *in vivo*. In this 24-hour model, the index AUC/MIC was most closely linked with efficacy. Notably, targets for AUC/MIC and T>Threshold were similar between organism groups. Enterobacteriaceae stasis was noted at AUC/MIC exposures of ~20 and maximal survival was observed at exposures similar to stasis endpoints.

2.3. Potential Risks and Benefits

2.3.1. Potential Risks

The oral and IV formulations of fosfomycin have been used for >45 years within and outside the US. As such, the potential risks for subject participation include procedures associated with the trial (e.g., blood sampling, ECG, and bronchoscopy) and/or those risks associated with well-characterized, low-incidence AEs that have followed IV fosfomycin administration.

IV fosfomycin has a high sodium load, which may result in hypokalemia and may require oral or IV potassium supplementation in some subjects. Serum electrolyte levels and water balance must be monitored during IV fosfomycin therapy. Caution is advised when IV fosfomycin is used in patients with cardiac insufficiency, hypertension, hyperaldosteronism, hypernatremia, or pulmonary edema.

Acute, potentially life-threatening hypersensitivity reactions (e.g., anaphylactic shock) may occur rarely. At the first signs of potential anaphylaxis (i.e., sweating, nausea, cyanosis), the infusion will be discontinued. Depending upon the clinical situation, appropriate emergency measures may need to be initiated.

Antibacterial agent-associated colitis and pseudo-membranous colitis have been reported with nearly all antibacterial agents including fosfomycin, and may range in severity from mild to life-threatening. Therefore, it is important to consider this diagnosis in patients who present with diarrhea during or after IV fosfomycin therapy.

The following AEs have been observed commonly or uncommonly following IV fosfomycin therapy in published literature or clinical trials, or are recognized in labeling outside the US:

- Common (≥1/100 to <1/10): nausea, vomiting, diarrhea, abdominal discomfort, injection-site phlebitis, liver enzyme abnormalities, QT interval prolongation, hypocalcemia, hypokalemia, hypophosphatemia, headache
- Uncommon (≥1/1,000 to <1/100): decreased appetite, hypernatremia, dysgeusia, vertigo, confusion, visual changes, dyspnea, rash, fatigue, edema,
- Rare (≥1/10,000 to <1/1000): aplastic anemia, eosinophilia
- Very rare (<1/10,000): fatty liver (completely reversible after drug discontinuation), anaphylaxis, visual impairment
- Unknown frequency: pseudomembranous colitis, hepatitis, cholestasis, tachycardia, asthma attack, allergic reaction (angioedema, pruritis, urticaria), agranulocytosis, granulocytopenia, leukopenia, pancytopenia, thrombocytopenia, neutropenia, confusion

The following potential risks are associated with other study procedures done as part of the trial:

- Associated with insertion of peripheral IV catheter and venous phlebotomy: momentary discomfort and bruising; infection, excess bleeding, clotting, or fainting are unlikely.
- Associated with ECG: skin irritation, itching, and redness from placement of ECG electrode pads.
- Associated with bronchoscopy: hemoptysis, epistaxis, local discomfort, fever, sore throat, respiratory distress, and respiratory failure. Sedative medications may be given as part of the bronchoscopy and may cause hypotension, lightheadedness, or dizziness; these side effects are somewhat common but very transient. Appropriate monitoring equipment and medications will be used to promptly identify and manage complications if they occur. Bronchoscopy may cause coughing and occasionally gagging. Fever or chills may occur, especially following BAL. Chest pain from atelectasis may occur. Some people experience temporary vomiting, and sore nose/throat. Mild-to-moderate cough may occur for up to 24 hours after the procedure.

Protection against risk: During the bronchoscopy, subjects will be placed on supplemental oxygen and monitored continuously for oxygen saturation and electrocardiogram changes. The topical anesthesia and sedation dosages will be monitored, and limits will be set and adhered to closely. Pre- and post-bronchoscopy care will be provided and will include adequate nursing staff and standard post-sedation recovery care with direct investigator oversight. To protect against the risk of QT prolongation, which could be caused by abnormalities in potassium, magnesium, calcium, and phosphorus, these electrolytes

will be measured prior to subjects undergoing the stress of bronchoscopy. Subjects with grade II or higher abnormalities in these electrolytes will not undergo bronchoscopy.

2.3.2. Known Potential Benefits

Given the nature of this single-dose trial in healthy subjects, there are no direct benefits to subjects outside of the laboratory screening and physical exam that each subject will receive. However, there is potential societal benefit. Participation in this study provides an assessment of the safety and PK of ZTI-01, enabling PK estimations and dosing identification to proceed into patients, including those requiring a new therapeutic option for difficult-to-treat infections caused by ESBL-producing and MDR bacteria.

3. OBJECTIVES AND OUTCOME MEASURES

3.1. Study Objectives

Safety:

- Determine the safety of multiple doses of ZTI-01 (6 g dose every 8 hours, administered as a 1-hour IV infusion, for a total of 18 g in three doses)

Pharmacokinetic:

- Determine the plasma and intrapulmonary PK (ELF and AM concentrations of ZTI-01) after multiple doses of ZTI-01 (6 g dose every 8 hours, administered as a 1-hour IV infusion, for a total of 18 g in three doses)

3.2. Study Outcome Measures

Safety:

The safety outcome measure will be the occurrence of adverse events (AEs), summary of physical exam findings, vital signs, clinical laboratory tests, and ECGs (Appendix B) at any time from the start of study drug administration through the end of subject follow-up.

All subjects who receive at least one dose of study drug will be included in the safety outcome measure. AEs will be collected from the time of dosing on Day 1 through the telephone follow-up interview on Day 3. TEAEs will be assessed by severity and relationship to the study drug as determined by the investigator.

Pharmacokinetics:

The pharmacokinetic outcome measures will be plasma and intrapulmonary concentrations of ZTI-01 by validated bioassays.

Plasma PK parameters will be derived from the plasma concentration vs. time curves after the third dose of study drug. The parameters include, but are not limited to:

- Maximum measured plasma concentration (C_{max})
- Area under the concentration vs. time curve (AUC_{0-8} and AUC_{0-inf})
- Time to peak concentration (t_{max})
- Terminal elimination half-life ($t_{1/2}$)

- Terminal-phase elimination rate constant (λ_z)
- Volume of distribution (Vd)
- Clearance (CL)

Intrapulmonary PK parameters will be derived from the ratios of drug concentrations in ELF and AMs to simultaneous drug concentrations in plasma for each subject and summarized for each sampling time. The median concentrations of fosfomycin from the BAL sampling times will be used to estimate the AUC_{0-8} of plasma, ELF, and AM. The ratio of AUC_{0-8} of ELF-to-plasma and AM-to-plasma will be calculated to determine the percent lung penetration.

4. STUDY DESIGN

This is a Phase 1, open-label, multiple-dose trial conducted at a single center. The treatment period will consist of three 6 g doses of ZTI-01 as a 1 h IV infusion (Table 1). A total of 30 enrolled subjects will be randomized. Ten additional enrolled subjects will act as alternates. An evaluable subject is defined as a subject who receives all doses of ZTI-01, undergoes BAL at the randomized sampling timepoint with BAL return volume adequate for testing, and undergoes at least the one blood sampling timepoint that is concurrent with the assigned BAL sampling timepoint, with blood sampling volume that is adequate for testing.

Each subject will complete Screening, Baseline, Treatment, and Follow-up Phases. The Screening Phase will be conducted on an outpatient basis within 30 days of the Baseline Phase. Baseline Phase will consist of admission to DEPRU on the day before the first dose (Day -1) for pre-dosing assessments. The Treatment Phase (Day 1 to Day 2) will include dosing 6 g of ZTI-01, administered as a 1-hour IV infusion (+10 minute window), every 8 hours for a total 18 g in 3 doses, followed by post-treatment safety assessments, and BAL and blood sample collections for PK measurements. Each subject will undergo a single standardized bronchoscopy with BAL after the start of the last IV infusion of ZTI-01 (Table 2). Blood samples for PK measurements will be collected before each dose, up to 8 hours after the start of the first IV infusion of ZTI-01, and up to 12 hours after the start of the third IV infusion (Table 3).

Subjects will remain in DEPRU during the Treatment Phase and will be discharged at least 12 hours after the start of the third IV infusion (if safety parameters are acceptable to the investigator). Safety assessments will be conducted during the entire Treatment Phase. The Follow-Up Phase will consist of a telephone interview on Day 3 for symptom-driven safety assessments.

5. STUDY ENROLLMENT AND WITHDRAWAL

Subjects will not be enrolled unless they meet the following inclusion and exclusion criteria. The investigator or other study personnel will document in the source documents (e.g., the clinical chart) that the informed consent form (ICF) was signed and dated before study screening. The time and date that informed consent was obtained will be recorded in the source documents and electronic case report form (eCRF). The presence of inclusion criteria and the absence of exclusion criteria will be recorded in the eCRF.

5.1. Subject Inclusion Criteria

To be considered for study enrollment, subjects must meet all of the following inclusion criteria:

1. Healthy[1] men and women aged 18-45 years with no clinically significant findings[2] at Screening and Baseline (Day -1 to Day 1)[2]

   [1]Healthy is defined by the absence of any medical condition described in the exclusion criteria in a subject with a normal physical exam including vital signs. If the subject has another current, ongoing medical condition, the condition cannot meet any of the following criteria: 1) first diagnosed within 3 months of enrollment; 2) is worsening in terms of clinical outcome in the last 6 months; or 3) involves need for medication.

   [2]Including findings on medical history, physical exam, vital signs, 12-lead ECG, or clinical laboratory tests.

2. Body Mass Index (BMI) = 18-30 kg/m^2, inclusive, and body weight >50 kg (110 lbs)

3. Females who have been surgically sterilized via bilateral oophorectomy and/or hysterectomy at least 90 days prior to Screening are considered lacking childbearing potential and will be eligible[3]

   [3]Postmenopausal females are not eligible, as the definition of menopause would require age >45, and all subjects in this study are age ≤45.

4. Females of childbearing potential must have a negative serum pregnancy test at Screening, a negative urine pregnancy test at Baseline (Day -1 to Day 1), and must use acceptable contraception[4]

   [4]Acceptable contraception methods are restricted to surgical sterilization (bilateral tubal ligation) or successful Essure placement (permanent, non-surgical, non-hormonal

sterilization with documented radiological confirmation at least 90 days after the procedure), use of long-acting reversible contraceptive devices (progestin-releasing subdermal implants [Nexplanon and Implanon, Merck], copper intrauterine devices [Paragard, Teva], and levonorgestrel-releasing intrauterine devices [Mirena, Bayer; Skyla, Bayer; Liletta, Allergan/Medicines360]), licensed hormonal products such as injectables or oral contraceptives, barrier methods such as condoms or diaphragms with spermicidal agents, and abstinence from sexual intercourse with a male partner.

Subjects must have used the above-listed method for a minimum of 30 days prior to the first dose of study drug and be willing to use the method for at least 30 days after the final dose of study drug.

5. Male subjects[5] whose partners are of childbearing age or pregnant must be willing to use condoms during the study and through the Day 3 follow-up call.

   [5]*including men who have had vasectomies*

6. Able to abstain from alcoholic beverages within 48 hours before Baseline (Day -1 to Day 1) and throughout the Treatment Phase

7. Able to abstain from caffeine use within 7 days before Baseline (Day -1 to Day 1) and throughout the inpatient period

8. Willing to remain in DEPRU during the Baseline and Treatment Phases

9. Have a high probability for compliance and completion of the trial

10. Sign a dated, witnessed, written ICF

11. Have adequate venous access for infusions and blood draws

5.2. Subject Exclusion Criteria

Subjects must meet none of the following exclusion criteria:

1. Any surgical or medical condition that in the opinion of the investigator could interfere with drug absorption, distribution, metabolism, or excretion.

2. Any surgical or medical condition that in the opinion of the investigator may place the subject at increased risk while participating in the trial.

3. History or presence of cardiovascular disease including coronary artery disease and chronic hypertension (systolic pressure >140 mmHg or diastolic pressure >90 mmHg)
4. Abnormal ECG at screening, as determined by the investigator to be clinically significant
5. History or presence of renal impairment or chronic renal disease
6. History or presence of liver disease (ALT, AST, or total bilirubin above the upper limit of normal)
7. History or presence of chronic pulmonary disease, including asthma, requiring use of medication in the year before screening
8. History of intolerance or hypersensitivity to phosphonic acid derivative antibiotics or any of its constituents (i.e., oral or IV fosfomycin)
9. Have cancer or have a history of cancer within the past 5 years, with the exception of non-melanomatous skin cancer, treated, without evidence of recurrence
10. Any medical condition that prevents a subject from undergoing bronchoscopy with BAL
11. Serum creatinine above the upper limit of normal, or estimated creatinine clearance (CrCl) <60 mL/min as determined by Cockcroft-Gault equation[6]

    [6]*Cockcroft-Gault equation where age is in years, weight is in kilograms, and serum Cr is in mg/dL units:*

    Males: CrCl (mL/min) = (140 – age) x Weight / (72 x Cr)

    Females: CrCl (mL/min) = [(140 – age) x Weight / (72 x Cr)] x 0.85
12. History of regular alcohol consumption within 6 months of Baseline (Day -1 to Day 1)[7]

    [7]*History of regular alcohol consumption is defined as an average weekly intake of >14 drinks for males or >7 drinks for females. One drink is equivalent to 12 g of alcohol: 12 ounces (360 mL) of beer, 5 ounces (150 mL) of wine, or 1.5 ounces (45 mL) of 80 proof distilled spirits.*
13. History of ≥10 pack-years smoking, or history of any nicotine use[8] in the 6 months before Baseline (Day -1 to Day 1) or positive urine cotinine screen at Baseline (Day -1 to Day 1)[9].

    [8]*Including cigarettes, pipe, cigar, chewing tobacco, nicotine patch*

    [9]*A positive urine cotinine at screening is allowed if negative at baseline*
14. History of illicit drug use within 6 months of Baseline (Day -1 to Day 1)[10]

[10]*Use of cannabinoids with 1 month of Baseline (Day -1 to Day 1) is excluded, but other use of cannabinoids within 6 months is permitted.*

15. Use of any prescription drugs, except acceptable contraception methods listed above, within 30 days of Baseline (Day -1 to Day 1)
16. Involvement in other investigational studies of any type (drugs, devices, procedures) within 30 days of Baseline (Day -1 to Day 1)
17. Blood or blood products donation within 30 days of Baseline (Day -1 to Day 1)
18. Planning egg or sperm donation any time before Day 3 follow-up call
19. Use of any non-prescription medications, vitamins, consumption >2 times/week of products containing genuine licorice, caffeine, or dietary or herbal supplements within 7 days of Baseline (Day -1 to Day 1)[11]

    [11]*Excluded from this list is intermittent use of acetaminophen at doses ≤2 g/day. Herbal supplements must be discontinued 7 days before the initial dose of study drug on Day 1.*
20. Presence of any acute illness, including febrile illness with temperature >37.8°C (>100.0°F), within 7 days of Baseline (Day -1 to Day 1)
21. Currently pregnant or breastfeeding as determined by subject report
22. Positive tests for human immunodeficiency virus (HIV) 1 and 2 antibodies, hepatitis B surface antigen (HBsAg), or hepatitis C virus (HCV) antibody
23. Positive urine drug or positive breathalyzer test for alcohol at Screening or Baseline (Day -1 to Day 1) or positive cotinine at Baseline (Day -1 to Day 1)
24. Weight loss or gain of >10% within 30 days of Baseline (Day -1 to Day 1)
25. Any laboratory value at screening or enrollment that is Grade 2 or more (Appendix B). A laboratory value that is Grade 1 will be allowed if not considered to be clinically significant by the investigator.[12]

    [12]*Excluded from this list of permissible Grade 1 laboratory values are ALT, AST, or Total bilirubin per Exclusion Criteria 6; serum creatinine per Exclusion Criteria 11; and serum electrolytes including sodium, potassium, calcium, phosphorus and magnesium.*
26. History of infection with SARS-CoV-2 (COVID-19) within 3 months of Baseline (Day -1 to Day 1) or unresolved symptoms of COVID-19.
27. Positive test for SARS-CoV-2 (COVID-19) at Screening or Baseline (Day -1 to Day 1) within 72 hours of admission

5.3. Treatment Assignment Procedures

5.3.1. Randomization Procedures

Subjects will be randomized to a single standardized bronchoscopy with BAL sampling timepoint upon study enrollment using an assignment schedule as follows: 30 subjects will be randomized 1:1:1:1:1 to each of the five BAL sampling timepoints (30 minutes, 75 minutes, 2 hours, 5 hours, or 8 hours after the start of the third IV infusion of ZTI-01). Up to ten (10) subjects will be enrolled as alternates. Alternates will arrive at DEPRU on Day -1 to Day 1 and will adopt the planned BAL sampling timepoint of any other enrolled subject who fails to present for check-in or who fails to meet inclusion/exclusion criteria on Day -1 to Day 1. If all scheduled subjects present for check-in and meet Day -1 to Day 1 inclusion/exclusion criteria, then alternates will not undergo any further study procedures at that time. In that case, alternates may be assigned to a new bronchoscopy time on a future date, alternate status for a future date, or as replacement subjects (see Section 5.3.4).

Subjects will be enrolled online using the enrollment module of AdvantageEDC^{SM} software. The randomization code will be prepared by DCC statisticians and included in the enrollment module for this study. AdvantageEDC^{SM} will assign each subject to a sampling timepoint after the demographic and eligibility data have been entered.

Instructions for use of the enrollment module are included in the AdvantageEDC^{SM} User's Guide. Manual back-up procedures and instructions are provided for use in case the site temporarily loses Internet access or the online enrollment system is unavailable.

5.3.2. Reasons for Withdrawal and Discontinuation of Study Product Administration

Subjects may voluntarily withdraw their consent for study participation at any time without penalty or loss of benefits to which they are otherwise entitled.

An investigator may also withdraw a subject from receiving the study drug for any reason. Follow-up safety evaluations will be conducted, if the subject agrees. If a subject withdraws or is withdrawn before completion of the study, the reason for this decision will be recorded in the eCRF. The reasons might include, but are not limited to the following:

- Subject no longer meets eligibility criteria
- Subject becomes noncompliant

- Subjects develops a medical disease or condition, or new clinical finding(s), for which continued participation, in the opinion of the investigator, might compromise the safety of the subject, interfere with the subject's successful completion of this study, or interfere with the evaluation of responses
- Subject is lost to follow-up
- Subject becomes pregnant

The investigator will be explicit regarding study follow-up (e.g., safety follow-up) that might be carried out despite the fact the subject will not receive further study drug. If the subject consents, every attempt will be made to follow all AEs through resolution. The procedures that collect safety data for the purposes of research will be included in the original ICF or the investigator may seek subsequent informed consent using an IRB/IEC-approved ICF with the revised procedures.

The investigator will inform the subject that data already collected will be retained and analyzed even if the subject withdraws from the trial.

5.3.3. Handling of Withdrawals and Discontinuation of Administration

Subjects who discontinue will be asked about the reason(s) for their discontinuation and about the presence of any AEs. If possible, they will be seen and assessed by an investigator(s). AEs will be followed to resolution and/or until deemed stabilized by the investigator. Subjects who discontinue may be subject to replacement as described in Section 5.3.4. If subjects are not evaluable and thus are replaced, their samples will be discarded. If the subject received any amount of study drug, they will be encouraged to follow up for safety.

5.3.4. Subject Replacement

Subjects who initiate the Treatment Phase but are not evaluable will be replaced.

An evaluable subject is defined as a subject who receives all doses of ZTI-01, undergoes BAL at the randomized sampling timepoint with BAL return volume adequate for testing, and undergoes at least the one blood sampling timepoint that is concurrent with the assigned BAL sampling timepoint, with blood sampling volume that is adequate for testing. Replacement subjects will undergo study procedures on a separate date.

5.3.5. Termination of Study

The trial may be terminated at any time at the discretion of the Sponsor, DMID. Other possible reasons for study termination include development of significant laboratory toxicities outside of those AEs characterized in the label and/or literature for the study drug. If the trial is prematurely terminated by the Sponsor, any regulatory authority, or the investigator for any reason, the investigator will promptly inform the study subjects and assure appropriate therapy or follow-up for the subjects, as necessary. The investigator will provide a detailed written explanation of the termination to the IRB/IEC.

6. STUDY INTERVENTION/INVESTIGATIONAL PRODUCT

6.1. Study Product Description

6.1.1. Acquisition

The non-US commercial drug product that will be used in the trial is a single vial of ZTI-01 disodium powder (4 g presentations with recrystallized succinic acid, in a Type 1 glass vials). Nabriva Therapeutics Inc. will provide DMID an adequate amount of ZTI-01 to support the trial, and will ship it to the following address at Fisher BioServices for storage and site distribution:

DMID-CMS

20439 Seneca Meadows Parkway

Germantown, MD 20876

Tel: (240) 477-1350

Fax: (240) 477-1360

E-mail: DMID.CMS@ThermoFisher.com

Sterile water for injection (WFI) will be the diluent and will be supplied by DMID through Fisher BioServices.

Study product will be shipped to DEPRU upon request and approval by NIAID.

6.1.2. Formulation, Packaging, and Labeling

ZTI-01

Each Type 1, glass vial of ZTI-01 contains sterile white powder consisting of 5.28 g of fosfomycin disodium (equivalent to 4 g of fosfomycin and 1.28 g of sodium) and the inactive ingredient: recrystallized succinic acid. Each gram of ZTI-01 contains 330 mg of sodium.

Vials will be labeled with protocol number, product identity – 4 g of ZTI-01 (fosfomycin for injection), lot number, expiry date, and the IND caution statement "New Drug – Limited by Federal Law to Investigational Use Only," a statement to store at controlled room temperature, and a statement that each vial is for intravenous, single use only.

WFI

The WFI, United States Pharmacopeia (USP) is nonpyrogenic and contains no bacteriostatic, antimicrobial agent, or added buffer. This product will be used to dilute the ZTI-01 and will be supplied as a single-dose vial.

6.1.3. Product Storage and Stability

Glass vials of ZTI-01 will be stored in a secure and locked research pharmacy, with limited access and available to appropriate study personnel only, at controlled room temperature of 20°C to 25°C (68°F to 77°F). If a temperature excursion outside of 15°C to 30°C (59°F to 86°F) occurs, the site will discontinue use of the vial(s) and notify the DMID Product Support Team (PST) and Nabriva within 24 hours of awareness of the excursion, according to the MOP.

The Investigator, or a designee (e.g., research pharmacist) will ensure that all study drugs are appropriately stored and in accordance with applicable regulatory requirements.

Sterile water for injection (WFI) will be stored at USP controlled room temperature 20°C to 25°C (68°F to 77°F).

6.2. Dosage, Preparation, and Administration of Study Drug

Preparation of ZTI-01 for IV infusion will be performed by DEPRU's Research Pharmacist according to the protocol-specific MOP. The procedure for reconstituting the vials with sterile water for injection (WFI) is presented in Table 5.

Table 5: ZTI-01 dose preparation guidelines

ZTI-01 (fosfomycin) dosage	Number of vials reconstituted with 20 mL of sterile WFI added to each vial	Volume of Sterile WFI to dissolve ZTI-01 powder	Volume of dissolved ZTI-01 to be added for infusion	Volume of WFI to be added for infusion	Total volume for infusion
6 g	2	40 mL (20 mL for each of two vials)	30 mL (15 mL from each of two vials)	170 mL	200 mL

Each 1 g dose of ZTI-01 contains 330 mg of sodium. Due to the salt load of the formulation, sterile WFI will be used to reconstitute each ZTI-01 vial and to prepare the 200 ml ZTI-01 infusion (Table 6).

A total of two 4 g vials are required to prepare the 6 g dose. Deliver 20 mL of sterile WFI from the syringe into each vial containing 4 g of ZTI-01 powder to dissolve the powder. Always use sterile WFI and aseptic technique to reconstitute the powder. A slight degree of warming occurs when the powder is dissolved upon reconstitution of the vial, due to an exothermic reaction. After reconstitution, the product

will be used to prepare the infusion solution as follows, and as further detailed in the MOP: Using aseptic technique, withdraw an equal amount (15 mL) from each reconstituted vial (a total volume of 30 mL from both vials). The solution that is left in each vial will remain "unused." Add the 30 mL of diluted ZTI-01 to 170 mL of sterile WFI to yield the 200 mL total volume for infusion. The storage for the infusion bag, before administration, is limited to 4 hours at room temperature (20-25°C) or 24 hours at refrigerated conditions (2 - 8°C).

Table 6: ZTI-01 (disodium fosfomycin) reconstitution with WFI

Dose	Preparation Steps
6 g	Step 1: Add 20 mL of sterile WFI to each of two vials of ZTI-01 powder Step 2: Draw 30 mL total from both vials of diluted ZTI-01. Note: Draw an equal amount (15 mL) from each dissolved vial; the 5 mL left in the vials remain "unused." Step 3: Add the 30 mL of dissolved ZTI-01 to 170 mL of sterile WFI to yield the 200 mL total volume for infusion.

Note: The pharmacy will need to use/reconstitute 2 vials for each 6 g dose.

ZTI-01 should always be administered as a 200 mL infusion over 1 hour (+10 minute window).

Note: Each 1 g dose of ZTI-01 contains 330 mg of sodium; a 6 g dose of ZTI-01 contains 1980 mg of sodium. Each subject receiving 3 doses per protocol will be administered 5940 mg of sodium.

The ZTI-01 6 g infusion solution will be infused intravenously over 1 hour (+10 minute window) using an IV infusion pump at a rate set to 200 mL/hr. A dose will be administered every 8 hours for 3 doses.

Administering sterile WFI alone (i.e., with no additives) may increase risk of hemolysis in some subjects due to hypotonicity. Although the IV infusion of sterile WFI alone is contraindicated, the addition of ZTI-01 to sterile WFI is appropriate and recommended for IV administration. The use of 0.9% NaCl (isotonic saline) for infusion with ZTI-01 will be avoided, as the resultant hyperosmolar solution may irritate the vein.

6.3. Modification of Study Drug for a Subject

None

6.4. Accountability Procedures for the Study Drug

Chain of custody will be documented between the on-site pharmacy and the clinical staff responsible for administering the study drug.

The Site Principal Investigator (PI) is responsible for the distribution and disposition of the study drug, and has ultimate responsibility for accountability. The PI may delegate to the Site Research Pharmacist responsibility for study drug accountability. The Research Pharmacist will be responsible for and will maintain and document logs of receipt, accountability, dispensation, storage conditions, and final disposition of study drug. Upon completion or termination of the trial, all unused and used vials of ZTI-01 will be disposed according to DEPRU's standard operating procedures (SOPs) after accountability. The investigator is responsible for ensuring that no study drug is disposed until it is fully accounted for by the monitor.

6.5. Assessment of Subject Compliance with Study Drug

Study staff will be present for and during all study drug administrations. Visual inspection by study staff of the complete infusion is expected and will be appropriately recorded in the eCRF. It is important to document that the entire 200 mL infusion is administered with each dose. All interruptions or premature terminations of infusion will be documented.

6.6. Concomitant Medications/Treatments

Medications to treat any AEs the subject experiences during the trial are permitted. All information on concomitant medications will be recorded on the subject's eCRF and will include the name of the procedure or drug and duration of the treatment (start/stop times), dosages, and route. The intermittent intake of acetaminophen (maximum 2 g/day) is permitted throughout the trial if approved by the Site PI. Subjects are required to refrain from the use of all prescription medications for at least 30 days before administration of the first dose of study drug on Day 1 until the last PK sample is taken. The consumption of beverages containing alcohol is prohibited for 48 hours before Baseline (Day -1 to Day 1) until completion of the Treatment Phase. The consumption of caffeine is prohibited for 7 days prior to Baseline (Day -1 to Day 1) and during the Treatment Phase. Herbal supplements and over-the-counter meds (except acetaminophen at doses ≤2 g/day) must be discontinued 7 days before the first dose of study drug until completion of the Treatment Phase. The use of illegal drugs is prohibited within the 6 months prior to Baseline (Day -1 to Day 1) and is not permitted while subjects are enrolled in this study. A urine drug screen will be performed at Screening. If a subject is unable to comply with the restrictions described above, the subject's continued participation in the trial will be re-evaluated by the investigator.

7. STUDY SCHEDULE

7.1. Screening (Day -30 to Day -1)

Subjects will be screened within 30 days before Enrollment/Baseline (Day -1 to Day 1). A sufficient number of individuals will be screened to identify 40 subjects for enrollment that fulfill all entry criteria in order to obtain 30 evaluable subjects at the end of the trial. After the subject has signed the IRB-approved ICF, the following evaluations will be performed and/or recorded in the eCRF:

- Demographics (date of birth, gender, and race/ethnicity)
- Complete medical history and prior medication history to include all drugs taken (including non-prescription and herbal products) within 30 days before screening procedures
- Physical exam to include the following organs and organ systems: vital signs (temperature, blood pressure, respiratory rate, heart rate, peripheral oxygen saturation), skin, head and neck, lungs, heart, liver, spleen, extremities, lymph nodes, and nervous system.
- Record history of alcohol (within prior 6 months) and tobacco use (lifetime)
- Height and weight to determine BMI
- Standard 12-lead ECG
- Laboratory tests (urine and blood specimens) as outlined in Appendix A
- Urine drug screen, urine cotinine, and alcohol test (breathalyzer)
- Serum pregnancy test for females of childbearing potential
- Collect birth control method and counsel subjects on acceptable contraceptive measures
- Counsel participants to avoid consumption of non-prescription medications, vitamins, licorice (in large amounts), caffeine or dietary or herbal supplements in the 7 days prior to confinement and to avoid consumption of beverages and foods containing alcohol for 48 hours prior to confinement
- Confirm all inclusion and exclusion criteria are fulfilled
- SARS-CoV-2 (COVID-19) test will occur within 72 hours prior to admission at the Screening Visit (Day -30 to Day -1) if Screening Visit occurs between (Day -3 to Day -2)

7.2. Enrollment/Baseline (Day -1 to Day 1)

Subjects will be admitted to DEPRU on Day -1 or Day 1 before the first dose of study drug on Day 1. After admission to DEPRU, the following Enrollment/Baseline tests and evaluations will be performed.

All Enrollment/Baseline tests and evaluations will be performed within 36 hours of randomization to a bronchoscopy time.

- Physical exam to include the following organs and organ systems: vital signs (temperature, blood pressure, respiratory rate, heart rate, peripheral oxygen saturation), skin, head and neck, lungs, heart, liver, spleen, extremities, lymph nodes, and nervous system
- Weight
- Review changes in the subject's medical history since screening
- Laboratory tests as outlined in Appendix A
- Urine pregnancy test for females of childbearing potential
- Counsel subjects on acceptable contraceptive measures
- Urine drug screen, urine cotinine, and alcohol breath test (breathalyzer)
- Review prior concomitant medication history since screening
- Record non-pharmacologic treatments/procedures within 72 hours before enrollment in the eCRF
- Confirm all inclusion and exclusion criteria are fulfilled
- Standard 12-lead ECG
- Randomize to a bronchoscopy time
- Place two peripheral IVs
- SARS-CoV-2 (COVID-19) test will occur within 72 hours prior to admission at the Baseline Visit (Day -1 to Day 1) if test was not administered during Screening Visit

7.3. Day 1 to Day 2

- Administer study drug to subjects every 8 hours for a total of 3 doses.
- Obtain vital signs, including heart rate, blood pressure, temperature, respiratory rate and peripheral oxygen saturation before each dose of study drug, and at approximately 0.5, 1, 2, and 5 hours after the start of each dose of study drug (T0 = time of each infusion start)
- Review and record any AEs and SAEs
- Review and record any concomitant medications, vitamins and herbal supplements
- Perform a standard 12-lead ECG within 2 hours after the third (last) dose of study drug

- Obtain PK blood samples at 15 timepoints: before (within 10 minutes) the first, second, and third doses of study drug, at 30 minutes (during infusion), 1 hour (within 5 minutes after the end of infusion), 1 hour 15 minutes, 2, and 5 hours from the start time of the first infusion of ZTI-01, and at 30 minutes (during infusion), 1 hour (within 5 minutes after the end of infusion), 1 hour 15 minutes, 2, 5, 8, and 12 hours from the start time of the third (last) infusion of ZTI-01
- Measurement of sodium, potassium, magnesium, calcium, and phosphorus approximately 2 hours prior to the assigned bronchoscopy timepoint
- A standardized bronchoscopy with BAL sampling will be performed at single timepoint based on each subject's schedule assignment at either 30 minutes, 1 hour 15 minutes, 2, 5, or 8 hours after the start time of the third (last) infusion of ZTI-01
- Collect a blood sample to measure serum urea concentration just before or during bronchoscopy

7.4. Day 2 (Day of Discharge from DEPRU)

- Laboratory tests and urinalysis as outlined in Appendix A
- Review and record any AEs and SAEs
- Review and record any concomitant medications, non-pharmacologic treatments or procedures
- Perform a physical exam to include the following organs and organ systems: vital signs (temperature, blood pressure, respiratory rate, heart rate, peripheral oxygen saturation), skin, head and neck, lungs, heart, liver, spleen, extremities, lymph nodes, and nervous system; measure weight before discharge

7.5. Day 3 (Telephone Follow-up Call)

A telephone call follow-up will be conducted the day following each subject's discharge from DEPRU to ask about subject's clinical status, concomitant medications, and AEs/SAEs. In the event of ongoing or new AEs/SAEs, subjects may be asked to return to DEPRU for evaluation at the investigator's discretion. Ongoing AEs/SAEs will be followed until resolved or stable/improving over two assessments and no further intervention is required.

7.6. Early Termination Visit

If the subject is prematurely discontinued from the trial, the same exit procedures as those performed on Day 1 or 2 will be performed before discharging the subject from DEPRU. If no clinical or laboratory abnormalities are observed, subjects are considered to have completed the protocol and will exit the trial. Additional follow-up visits will be scheduled if a subject has an ongoing clinically significant abnormality at this visit and/or the clinical laboratory evaluations reveal a clinically significant laboratory abnormality, as deemed appropriate by the investigator or designee. Clinically significant laboratory

abnormalities will be monitored periodically until resolution or until they are considered irreversible, or until there is a satisfactory explanation for the changes observed.

7.7. Unscheduled Visit (if needed)

- Review and record any AEs and/or SAEs
- Review and record any concomitant medications, non-pharmacologic treatments or procedures
- Perform a physical exam to include the following organs and organ systems: vital signs (temperature, blood pressure, respiratory rate, heart rate, peripheral oxygen saturation), skin, head and neck, lungs, heart, liver, spleen, extremities, lymph nodes, and nervous system
- Obtain a standard 12-lead ECG at the investigator's discretion
- Other studies as clinically indicated at the investigator's discretion

8. STUDY PROCEDURES/EVALUATIONS

8.1. Clinical Evaluations

Medical history: history or presence of prior and current medical conditions or significant surgical procedures

Physical exam: overall physical assessment (to include vital signs [temperature, blood pressure, respiratory rate, heart rate, peripheral oxygen saturation], skin, head and neck, lungs, heart, liver, spleen, extremities, lymph nodes, and nervous system) and weight will be collected at Screening, Baseline (Day -1 to Day 1) and Day 2 (day of discharge). Height will be measured and BMI calculated at screening only.

ECG: performed in the supine or semi-recumbent position after resting for at least 10 minutes at 3 timepoints: Screening, Baseline (Day -1 to Day 1), and Day 2 within 2 hours of the third (last) dose of study drug. Each ECG will be interpreted and abnormal results acted upon by the investigator as outlined for each timepoint (Screening and Baseline ECG abnormalities per Exclusion Criteria, Day 2 ECG abnormalities per Appendix B). In case of premature discontinuation, ECG will be performed before discharge.

Vital signs: heart rate, blood pressure, temperature, respiratory rate, and peripheral oxygen saturation will be measured with the subject in the supine or semi-recumbent position, will be obtained at the following timepoints: Screening, Baseline (Day -1 to Day 1), before each of 3 doses of study medication, approximately at 0.5, 1, 2, and 5 hours after the start of each infusion of study drug, and before discharge from DEPRU on Day 2. Since changes in vital signs are expected during bronchoscopy, when a vital sign timepoint is scheduled during a subject's bronchoscopy, that timepoint will be measured immediately before the start of bronchoscopy. If vital signs are abnormal, repeat readings will be obtained up to twice more (total of 3 measurements including the first one), 5-10 minutes apart. If they remain abnormal, unscheduled vital signs will be repeatedly obtained until they normalize (at investigator's discretion).

Prior/Concomitant Medications/Treatments: information regarding allergies and use of prescription medications, non-prescription medications, herbal supplements, nicotine, caffeine, alcohol, and illicit drugs will be obtained at Screening, Baseline (Day -1 to Day 1), Treatment (Days 1-2), Day 2 before discharge, and throughout each subject's enrollment as required.

8.2. Laboratory Evaluations

8.2.1. Clinical Laboratory Evaluations

Serum chemistry: albumin, glucose, blood urea nitrogen (BUN), potassium, magnesium, calcium, sodium, total protein, creatinine, triglycerides, total cholesterol, creatine phosphokinase (CPK), phosphorus, alkaline phosphatase (ALP), AST, ALT, total bilirubin, direct bilirubin, and lactate dehydrogenase (LDH) will be collected at Screening and Day 2 before discharge. Albumin, glucose,

BUN, potassium, magnesium, calcium, sodium, phosphorus, total protein, creatinine, CPK, ALP, AST, ALT, and total bilirubin will be collected at Baseline (Day -1 to Day 1). Sodium, potassium, magnesium, calcium, and phosphorus will be collected approximately 2 hours prior to the assigned bronchoscopy timepoint.

Hematology: CBC with differential (hemoglobin, hematocrit, platelet count, white blood cell (WBC) count, neutrophils, lymphocytes, monocytes, eosinophils, basophils) will be collected at Screening and Day 2 before of discharge.

Coagulation: PT and aPTT will be collected at Screening and Day 2 before discharge.

Urinalysis: leukocyte esterase, blood, pH, specific gravity, glucose, protein. A microscopic test will be performed if urinalysis is abnormal. The microscopic urinalysis result for the presence of blood will supersede the dipstick urinalysis result. Urinalyses will be performed at Screening and Day 2 before discharge.

Serum and urine pregnancy test: a serum pregnancy test will be performed on all females of childbearing potential at Screening, a urine pregnancy test will be performed on all females of childbearing potential at Baseline (Day -1 to Day 1).

Test for SARS-CoV-2 (COVID-19) at Screening or Baseline (Day -1 to Day 1) within 72 hours of admission

Screening for Chronic Infections: HIV antibody, HBsAg, and HCV antibody will be measured at screening

Alcohol (breathalyzer) test, urine cotinine, and urine drug testing: performed at Screening and Baseline (Day -1 to Day 1). Urine will be collected and tested separately for cotinine and illicit drugs: barbiturates, benzodiazepines, THC, cocaine, opiates, and amphetamine/methamphetamine.

Urea: a blood sample for plasma urea concentration will be obtained just before or during each subject's scheduled bronchoscopy.

8.2.2. Bronchoscopy and Bronchoalveolar Lavage (BAL)

Each subject will be assigned to one bronchoscopy sampling timepoint. The bronchoscopy sampling times will be at 30 minutes, 1 hour 15 minutes, 2, 5, and 8 hours from the start time of the third (last) infusion of ZTI-01. A total of 6 subjects will be assigned to each of the five BAL sampling times.

Sodium, potassium, magnesium, calcium, and phosphorus will be measured approximately 2 hours prior to each subject's bronchoscopy timepoint. Subjects with grade 2 or higher abnormalities (Appendix B) in these electrolytes will not receive any further study product and will not undergo bronchoscopy.

Bronchoscopy will be performed in a standardized fashion according to the MOP. Topical lidocaine will be applied to the upper airways to prepare subjects for bronchoscopy. If needed, 1.0% percent lidocaine

will be used in the lower airway. A fiberoptic bronchoscope will be inserted into the right middle lobe. Four 50-mL aliquots of sterile normal saline will be instilled into the right middle lobe and each specimen will be immediately aspirated and placed on ice. BAL samples for PK measures will be obtained ±15 minutes from the subject's assigned sampling time. The start and stop times of BAL will be recorded on the eCRF.

Subjects will be continuously monitored during bronchoscopy. Blood pressure, heart rate, respiratory rate, and peripheral oxygen saturation will be recorded before and at the end of bronchoscopy. Subjects will undergo cardiac monitoring during bronchoscopy.

8.2.3. Pharmacokinetic Blood and BAL Samples

An assay to measure ZTI-01 concentrations in plasma, BAL, and cell pellet samples and urea in plasma and BAL samples will be conducted at a central analytical lab using a validated LC-MS/MS bioanalytical assay. Timing of samples will be with respect to the start of each dose of study drug (pre-dose and post-infusion start, when applicable). The date and time of each PK sample and BAL collection will be recorded in the eCRF. Blood PK samples will be drawn for all subjects within the following windows: -10 to -1 minute for scheduled samples at pre-dose; ±5 minutes for scheduled samples at 30 minutes, 1 hour 15 minutes, and 2 hours; +0-5 minutes from end of infusion for the scheduled sample at 1 hour; and ±15 minutes for scheduled samples at 5, 8, and 12 hours. BAL samples for PK (fosfomycin and urea assays) will be obtained within +/- 15 minutes of the assigned sampling time.

Instructions for collecting, processing, storage, and shipping of plasma, BAL, and urine samples will be provided in the MOP.

Blood and BAL samples are summarized in Table 7.

Table 7: Pharmacokinetics sampling times

Study day	Dosing	Blood sampling times*	BAL sampling times§
Day 1-2 Dosing	First dose: ZTI-01 6 g IV infusion over 1 hour (+10 minute window)	All subjects: Pre-dose (-10 to -1 minutes), 30 minutes (during the 1-hour infusion), and 1 hour, 1 hour 15 minutes, 2, and 5 hours after the start of the first dose	No sampling
	Second dose: ZTI-01 6 g IV infusion over 1 hour (+10 minute window)	All subjects: Pre-dose (-10 to -1 minutes)	No sampling
	Third dose: ZTI-01 6 g IV infusion over 1 hour (+10 minute window)	All subjects: Pre-dose (-10 to -1 minutes), 30 minutes (during the 1-hour infusion), and 1 hour, 1 hour 15 minutes, and 2, 5, 8, and 12 hours after the start of the third (last) infusion of ZTI-01	Each subject: Complete a single bronchoscopy with BAL at one of the five sampling times: 30 minutes (during the 1-hour infusion), 1 hour 15 minutes, 2, 5, or 8 hours from the start of the third (last) infusion of ZTI-01
Day 2 At Discharge	No further dosing	All subjects: 12 hours after the start of the third (last) infusion of ZTI-01	No sampling

* Blood PK samples will be drawn for all subjects within the following windows: -10 to -1 minutes for scheduled samples at pre-dose; ±5 minutes for scheduled samples between 30 minutes, 1 hour 15 minutes, and 2 hours; +0-5 minutes from end of infusion for the scheduled sample at 1 hour; ±15 minutes for scheduled samples at 5, 8, and 12 hours. Actual times for dosing and sample will be recorded.

§ BAL samples for PK (fosfomycin and urea) will be drawn ±15 minutes for scheduled samples. Actual times for dosing and sampling will be recorded. A blood sample will be collected to measure serum urea concentration just before or during the bronchoscopy.

8.2.4. Specimen Preparation, Handling, and Shipping

Please reference the protocol-specific MOP for detailed instruction for collection, processing, labeling, storing, handling and shipping.

8.2.4.1. Instructions for Specimen Preparation, Handling, and Storage

All samples collected and processed for PK analysis will be shipped in accordance with the MOP to DMID-CMS at the address below, and will be tracked using DMID's Global Trace Sample Tracking system.

DMID-Clinical Materials Services (Fisher BioServices)

20439 Seneca Meadows Parkway

Germantown, MD 20876

Tel: 240-477-1350,

Fax: 240-477-1360

Email: dmid.cms@thermofisher.com

Blood samples: For each blood sample timepoint (according to Sections 8.2.1 and 8.2.3), approximately 4 mL of blood will be drawn into a vacutainer tube and will be processed according to the MOP.

BAL samples: BAL samples will be collected according to Section 8.2.2 and processed for PK and urea measurements according to the MOP.

Specimen shipment: Plasma and BAL cell pellet samples will be shipped overnight on dry ice to DMID-CMS, which will then send one set of samples to the bioanalytical lab performing the PK analysis. See the MOP for details for shipping.

9. ASSESSMENT OF SAFETY

9.1. Specification of Safety Parameters

All DMID studies are conducted in accordance with 45 CFR 46, which provides for the protection of study subjects. The Site PI or designee is responsible for the safe conduct of the trial and for reporting AEs and SAEs according to the protocol and applicable federal regulations. An Independent Safety Monitor (ISM) is a physician experienced in the conduct of clinical studies, located close to DEPRU, and appointed to independently review all SAEs and related Grade 3 AEs and thoroughly investigate those considered both serious and unexpected. The DMID Medical Monitor reviews progress and safety reports, reviews safety reports prepared in advance for SMC meetings and comments on their accuracy, reviews SMC recommendations, and has the authority to halt, suspend, or stop the study.

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

9.2.1. Adverse Events

ICH E6 defines an AE as any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product, and that does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including a clinically significant laboratory abnormality), symptom, or disease temporally associated with use of the study drug, whether or not it is deemed related to the study drug. The occurrence of an AE may come to the attention of study personnel during study visits and interviews of a study subject presenting for medical care, or upon review by a study monitor. The period of observation for AE reporting will begin on Day 1 at the time of first dosing and continue through the final visit, a follow-up call on Day 3.

All AEs including local and systemic reactions not meeting the criteria for SAEs will be captured on the appropriate eCRF. Information to be collected includes event description, time of onset, clinician's assessment of severity, relationship to study drug (assessed only by those with the training and authority to make a diagnosis), outcome, action taken, and time of resolution/stabilization of the AE. All AEs occurring while on study will be documented appropriately regardless of relationship. All AEs will be followed until they adequately resolve or are determined by the PI to be medically stable (stable or improving over two assessments and no further intervention required). Any medical condition that is present at the time that the subject is screened will be considered as a baseline finding and not reported as an AE. However, if its Grade increases at any time during the trial such that it meets the AE definition specified above, it will be recorded as an AE.

All systemic and laboratory AEs will be graded using the Adverse Event Toxicity Grading Scale (Appendix B). Laboratory toxicity grading will be determined by using these tables as modified for the study to accommodate the reference range of the Central Laboratory and the acceptable ranges used in

determining eligibility. If a subject was enrolled in the trial with a laboratory value that is outside the reference range, but within the acceptable ranges, , an AE will be recorded if it otherwise meets the definition of AE in Section 9.3 and the on-study value is higher (if initially high) or lower (if initially low) than the screening value.

Severity of Event:

All AEs will be assessed by the clinician using the protocol-defined grading system in Appendix B.

Changes in the severity of an AE will be documented to allow an assessment of the duration of the AE at each level of intensity to be performed. AEs characterized as intermittent require documentation of onset and duration of each episode.

Relationship to Study Products:

The clinician's assessment of an AE's relationship to the study drug is part of the documentation process, but it is not a factor in determining what is or is not reported in the trial. If there is any doubt as to whether a clinical observation is an AE, it will be reported. All AEs will have their relationship to the study drug assessed using the terms "related" or "not related." In a clinical trial, the study drug must always be suspect. To help assess relatedness, the following guidelines are used.

- Related: There is a reasonable possibility that the study drug caused the AE. Reasonable possibility means that there is evidence to suggest that the study drug caused the AE.
- Not Related: There is not a reasonable possibility that the study drug caused the AE.

9.2.2. Serious Adverse Events

An AE or suspected AE is considered "serious" if, in the view of either the investigator or Sponsor, it results in any of the following outcomes:

- Death,
- a life-threatening AE,
- An adverse event is considered life-threatening if, in the view of either the site principal investigator or sponsor, its occurrence places the patient or subject at immediate risk of death. It does not include an adverse event that, had it occurred in a more severe form, might have caused death.
- inpatient hospitalization or prolongation of existing hospitalization,
- a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or
- a congenital anomaly/birth defect.

- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious 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.

All SAEs will be:

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

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

A licensed study physician will make the determination of seriousness, severity, and causality, provide a medical evaluation of adverse events, and classify adverse events based upon medical judgment. Abnormal laboratory test values or abnormal clinical findings for all enrolled subjects, including values and findings noted at screening and baseline, will be assessed using the toxicity scales in Appendix B. Abnormal values and findings noted at Screening or Baseline will only be considered AEs if they increase in grade after study product administration. For abnormalities noted from the time of study drug administration, any Grade 1 or higher laboratory abnormality listed on the toxicity table in Appendix B will be entered in the database as an AE. Abnormal clinical/laboratory findings will be collected, assessed, documented, reported, and followed appropriately.

9.3. Reporting Procedures

AEs will be reported from the time of dosing on Day 1 through the Telephone Follow-up Interview on Day 3.

All AEs will be followed until resolution. 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 AEs and SAEs will be reported, whether or not that are deemed causally related to the study drug. All AEs and SAEs will be recorded in the eCRF.

SAEs will be followed until resolution even if this extends beyond the study-reporting period.

The Site PI is responsible for informing the IRB of the SAE as per local requirements. DMID will be responsible of informing the US Regulatory Authority of the SAE as per federal requirements.

9.3.1. Serious Adverse Events

Any AE that meets a protocol-defined serious criterion will be submitted immediately (within 24 hours of site awareness) on an SAE form to the DMID Pharmacovigilance Group, at the following address:

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 form, select SAE data fields will also be entered into the DCC system (e.g., Advantage EDC). Please see the protocol-specific MOP for details regarding this procedure.

Other supporting documentation of the SAE may be requested by the DMID Pharmacovigilance Group and will be provided as soon as possible.

DEPRU will send a copy of the SAE report(s) to the ISM when they are provided to the DMID Pharmacovigilance Group. The DMID Medical Monitor and DMID Clinical Project Manager will be notified of the SAE by the DMID Pharmacovigilance Group. The DMID Medical Monitor 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 study, if the Site PI or appropriate sub-investigator becomes aware of an SAE that is suspected to be related to the study drug, the Site PI or appropriate sub-investigator will report the SAE to the DMID Pharmacovigilance Group.

9.3.2. Regulatory Reporting for Studies Conducted Under DMID-Sponsored IND

Following notification from the investigator, the IND sponsor DMID will report any suspected AE that is both serious and unexpected. DMID will report an AE as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the study drug and the AE. DMID will notify FDA and all participating investigators (i.e., all investigators to whom the Sponsor is providing drug under its INDs or under any investigator's IND) in an IND safety report of potential serious risks from clinical trials or any other source, as soon as possible, but in no case later than 15 calendar days after the Sponsor determines that the information qualifies for reporting as specified in 21 CFR Part 312.32. DMID will

also notify FDA of any unexpected fatal or life-threatening suspected AE as soon as possible but in no case later than 7 calendar days after the Sponsor's initial receipt of the information. Relevant follow-up information to an IND safety report will be submitted as soon as the information is available. Upon request from FDA, DMID will submit to FDA any additional data or information that the agency deems necessary, as soon as possible, but in no case later than 15 calendar days after receiving the request.

All SAEs designated as "not related" to the study drug will be reported to the FDA at least annually in a summary format.

9.3.3. Reporting of Pregnancy

Pregnancies occurring in study subjects will be reported via AdvantageEDC^{SM} on the Pregnancy Report form. All pregnancies occurring during the trial will be followed for information regarding the course of pregnancy and any outcome, delivery and condition of the newborn. Follow-up will be provided by the investigator to the Medical Monitor (or designee) in a timely manner.

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

All AEs will be followed until resolution or until determined by the PI to be medically stable (stable/improving and no further intervention required).

9.5. Halting Rules

If any of the halting rules, as outlined below, is met, further enrollment and dosing will be withheld until the SMC reviews the safety data and provides its recommendations.

- Two subjects experience any related Grade 3 systemic AE
- Two subjects experience the same related Grade 3 laboratory AE (per Medical Dictionary for Regulatory Activities High Level Group Term)
- One subject experiences a related SAE

A decision to reinitiate the trial and proceed with study drug administration will be made by DMID based on the recommendation of the SMC.

If the following individual halting rule is met, the individual will not undergo further dosing or study procedures.

- Subject experiences a grade 2 or higher abnormality in sodium, potassium, magnesium, calcium, or phosphorus, when checked approximately 2 hours prior to the bronchoscopy timepoint

9.6. Safety Oversight

The safety oversight for the trial will be conducted under the direction of DMID.

9.6.1. Independent Safety Monitor (ISM)

An ISM will oversee the safety of subjects and provide independent evaluation of all SAEs and related Grade 3 AEs. The ISM will communicate with the Site Investigators to discuss subject-related issues and resolve queries generated by the ISM, DMID Medical Monitor, and SMC, as applicable.

9.6.2. Safety Monitoring Committee (SMC)

An SMC will be appointed to monitor subject safety. The SMC will consist of independent evaluators who will have no relationship with the conduct of the trial. The SMC will operate under the rules of a DMID-approved charter that will be written at the organizational meeting of the SMC. In the charter, meeting frequency and each data element that the SMC will assess will be clearly defined.

The SMC will review safety data for increased rate of occurrence of serious suspected AEs. If halting rules are met, more frequent meetings may be held. Ad hoc meetings may also be held to address any safety issues that may arise.

If the trial is halted, the SMC will be consulted to review the data and provide a recommendation related to the continued dosing of the subjects.

The SMC will advise DMID of its recommendations. At the end of study enrollment, the SMC will review clinical safety and PK data, and provide recommendations regarding further safety assessments in studies with ZTI-01. Since the study is an open-label trial, a statistician may provide independent analyses to the SMC as needed.

10. CLINICAL MONITORING

Site monitoring of the trial will be conducted to ensure the safety and conduct of the trial complies with 21 CFR 11, 21 CFR 50, 21 CFR 54, 21 CFR 56, 45 CFR 46, 21 CFR 312 GCP and ICH Guidelines, and DMID guidelines, as appropriate.

10.1. Site Monitoring Plan

Site monitoring is conducted to ensure that the human subjects' protections, study and laboratory procedures, study intervention administration, and data collection processes are of high quality and meet Sponsor, ICH/GCP guidelines, and applicable regulations, and that the trial is conducted in accordance with the protocol, protocol-specific MOP, and applicable Sponsor SOPs. DMID, the Sponsor, or its designee will conduct site-monitoring visits as detailed in the clinical monitoring plan.

Site visits will be made at standard intervals as defined by DMID and may be made more frequently as directed by DMID. Monitoring visits will include, but are not limited to, review of regulatory files, accountability records, eCRFs, ICFs, medical and laboratory reports, and protocol and GCP compliance. Site monitors will have access to DEPRU, 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 will document site visit findings and discussions.

11. STATISTICAL CONSIDERATIONS

11.1. Study Hypotheses

The objectives of the study are to assess safety and pharmacokinetics for a multiple dose regimen of IV-infused ZTI-01. The study is not designed to test any formal hypotheses, but will provide exposure information on the IV formulation of ZTI-01. These exposure data should act as a bridge between the new IV formulation and the approved oral reference drug.

These data could permit the application of previously submitted information (e.g. safety, pharmacology, toxicology, genotoxicity, carcinogenicity) in support of the new IV formulation.

This study will also provide adequate information to confirm the safety and PK of ZTI-01 and further evaluate its use in the intended patient population.

11.2. Sample Size Considerations

Since statistical analyses are not intended to serve as a basis of definitive conclusions, statistical power considerations for determination of sample size were not performed. The sample size for this study was selected as adequate for determining the safety and PK of ZTI-01. The sample size is based on clinical experience and judgment relative to study design and objectives. The study is designed to enroll 40 healthy male and female adult subjects, aged 18 to 45 years, to have 30 evaluable subjects complete the trial. This will result in 30 plasma and BAL concentration-time profiles available for PK profiling. Table 8 displays probabilities of detecting at least one safety event for a range of true event rates using the specified sample size of 30 subjects. Administration of ZTI-01 to any alternate subjects may result in a sample size higher than 30 for safety analyses. In this case, the probability of observing safety events will be higher than displayed in Table 8.

Table 8: Probability of observing at least one event for a range of true event rates based on a sample size of n=30 subjects

True Event Rate	Probability of observing 1 or more events
0.1%	3%
0.5%	14%
1%	26%
2%	45%
5%	79%
7%	89%
10%	96%
15%	99%

11.3. Final Analysis Plan

Analysis Populations:

All subjects who receive any amount of ZTI-01 will be included in the safety population. The pharmacokinetic analyses will be performed using the population of evaluable subjects. An evaluable subject is defined as a subject who receives all doses of ZTI-01, undergoes BAL at the randomized sampling timepoint with BAL return volume adequate for testing, and undergoes at least the one blood sampling timepoint that is concurrent with the BAL sampling timepoint, with blood sampling volume that is adequate for testing.

Safety:

The safety outcome measure will be the occurrence of AEs (Appendix B) at any time from the start of study drug administration through the end of subject follow-up.

All subjects in the safety population will be included in the safety analyses. AEs will be collected from the time of dosing on Day 1 through the Telephone Follow-Up Interview on Day 3. TEAEs will be tabulated by severity (mild, moderate, or severe), type (MedDRA system organ class and preferred term), and by the relationship to the study drug as assessed by the investigator. TEAEs will be summarized both by incidence and frequency. For summaries by incidence, subjects will be tabulated according to the most severe grade of AE experienced (each subject counted only once per type of AE). For summaries of

frequency, each AE (by type) will be tabulated by severity, with each occurrence of the AE being counted (same subject can contribute multiple events to frequency). Tabulations of incidence and frequency will be performed separately for AEs overall and for related AEs only.

Observed and change-from-baseline vital signs, laboratory, and ECG data will be summarized using the mean, median, standard deviation, minimum, and maximum. The most recent measurement prior to start of infusion of the first ZTI-01 dose will be regarded as baseline. The number and percentage of subjects with laboratory, vital sign, and ECG abnormalities at baseline, subsequent visits, or early termination from the trial will be tabulated. The results of all laboratory tests, physical exam findings, ECGs, and vital signs will be presented in data listings.

Pharmacokinetics:

The pharmacokinetic outcome measures will be plasma and intrapulmonary PK of ZTI-01.

The following plasma PK parameters will be derived from the plasma concentration vs. time curves for fosfomycin after ZTI-01 administration. Noncompartmental analysis will be used to determine PK parameters, but other modeling techniques can be employed as needed. The below PK parameters will be estimated using Phoenix® WinNonlin® version 8.0 or later, and using plasma concentrations collected after start of the first and/or third doses.

- Maximum measured plasma concentration (C_{max})
- Observed and extrapolated area under the concentration versus time curve (AUC_{0-8}; AUC_{0-12}; AUC_{0-inf})
- Time to peak concentration (t_{max})
- Terminal elimination half-life ($t_{1/2}$)
- Terminal-phase elimination rate constant (λ_z)
- Volume of distribution (Vd)
- Clearance (CL)

PK parameters (C_{max}, t_{max}, and AUC_{0-8}) will be compared by dose and the accumulation ratio will be estimated using both C_{max} and AUC_{0-8}.

Intrapulmonary PK of ZTI-01 will be derived as follows. Serum and BAL urea measurements will be used to estimate the volume of ELF recovered in the BAL samples. The ratios of ELF and AM concentrations of fosfomycin to simultaneous plasma concentrations will be calculated for each subject and summarized for each sampling time. The median concentrations of fosfomycin from the BAL sampling times will be used to estimate the population average AUC_{0-8} of plasma, ELF, and AM. The

ratio of population average AUC_{0-8} of ELF-to-plasma and AM-to-plasma will be calculated to determine the percent penetration.

Specific details of pharmacokinetic analysis will be included in the study-specific pharmacokinetic analysis plan.

12. SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA/DOCUMENTS

DEPRU will maintain appropriate medical and research records for this study, in compliance with Section 4.9 of ICH E6 GCP, and regulatory and institutional requirements for the protection of confidentiality of subjects. As part of participating in a DMID-sponsored study, DEPRU will permit authorized representatives of DMID and regulatory agencies to examine (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.

Source data are all information, original records of clinical findings, observations, or other activities in a 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, evaluation checklists, 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, and subject files and records kept at the pharmacies, laboratories, and medico-technical departments involved in the trial.

13. QUALITY CONTROL AND QUALITY ASSURANCE

Following a written DMID-accepted site quality management plan, DEPRU and its subcontractors are responsible for conducting routine quality assurance (QA) and quality control (QC) activities to internally monitor study progress and protocol compliance. The Site PI will provide direct access to all study-related sites, source data/data collection forms, and reports for monitoring and auditing by the Sponsor, and inspection by local and regulatory authorities. The Site PI will ensure all study personnel are appropriately trained and applicable documentations are maintained at DEPRU.

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 DEPRU for clarification and resolution.

14. ETHICS/PROTECTION OF HUMAN SUBJECTS

14.1. Ethical Standard

The Site 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 25691 (1997), if applicable. The Site PI's institution will hold a current Federalwide Assurance (FWA) issued by the Office of Human Research Protection (OHRP) for federally-funded research.

14.2. Institutional Review Board

Before enrollment of subjects into the trial, the protocol and ICF will be reviewed and approved by the appropriate IRB listed on its FWA.

The responsible official for the IRB will sign the IRB letter of protocol approval before the start of the trial and provide a copy and the IRB FWA number to DMID.

If amendments to the protocol are required, they will be written by the Sponsor and provided to the Site PI for submission to the IRB.

14.3. Informed Consent Process

The Site PI will choose subjects in accordance with the eligibility criteria detailed in Section 5.1. Before any study procedures are performed, subjects must sign an ICF that complies with the requirements of 21 CFR 50, 45 CFR 46, and the local IRB. Study personnel may employ recruitment efforts before obtaining consent if a subject-specific screening consent is on record or if the IRB has agreed that chart review is allowed without a fully executed screening consent. If there is no subject-specific screening consent on record, DEPRU clinical staff may pre-screen via chart review and refer potential subjects to the research staff. Research staff would obtain written consent per the standard informed consent process before conducting protocol-specific screening activities.

Informed consent is a process that is initiated before an individual agrees participate in a trial and continues throughout the subject's trial participation. Before any study procedures are performed, including pre-screening of subjects for eligibility, subjects will receive a comprehensive explanation of the proposed study procedures and study drug. This will include the nature, risks, and possible benefits of the trial, alternate therapies, any known AEs, the investigational status of the study drug, and other elements that are part of obtaining proper informed consent. Subjects will also receive a detailed explanation of the proposed use and disclosure of their protected health information, including specifically their blood, urine, and BAL samples. Subjects will be allowed sufficient time to consider participation in the trial, after having the nature, risks, and possible benefits of the trial explained to them,

and have the opportunity to discuss the trial with their family, friends, or legally authorized representative, or think about it before agreeing to participate.

ICFs describing in detail the study drug, procedures, risks, and possible benefits will be given to subjects. The ICF will not include any exculpatory statements. ICFs will be IRB-approved and subjects will be asked to read and review the appropriate document. Upon reviewing the appropriate document, the Site PI or designee will explain the trial to subjects and answer any questions that they have. Subjects must sign the ICF, and written documentation of the informed consent process is required before starting any study procedures being done specifically for the trial, including determining eligibility and administering study drug.

By signing the ICF, subjects agree to complete all study procedures required by the trial, unless the subject withdraws voluntarily, or is withdrawn or terminated from the trial for any reason. The rights and welfare of subjects will be protected by emphasizing to subjects that the quality of their medical care will not be adversely affected if they decline to participate in or withdraw from the trial.

DMID will provide the Site PI, in writing, any new information that significantly impacts the subject's risk of receiving the study drug. This new information will be communicated by the Site PI to subjects who consent to participate in the trial in accordance with IRB requirements. The ICF will be updated and subjects will be re-consented per IRB requirements, if necessary. Subjects will be given a copy of all ICFs that they sign.

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

The trial will enroll all subjects aged 18 to 45 years who meet all the Subject Inclusion Criteria (see Section 5.1.1) and do not meet any of the Subject Exclusion Criteria (see Section 5.1.2), regardless of religion, sex, race, or ethnicity. Persons younger than 18 years of age will not be included in this study. This trial is being performed to learn about the pulmonary pharmacokinetics of fosfomycin in healthy adults. The additional risks of performing bronchoscopy in pediatric populations warrants the exclusion of children in this trial.

14.5. Subject Confidentiality

Subject confidentiality is strictly held in trust by the Site PI, other study personnel, the Sponsor, and their agents. This confidentiality is extended to cover testing of biological samples, in addition to the clinical information relating to subjects. Subjects will be identified by code numbers and not by name.

The study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the trial or the data will be released to any unauthorized third party without prior written approval of the Sponsor.

All information provided by the Sponsor and all data and information generated by DEPRU as part of the trial (other than a subject's medical records) will be kept confidential by the Site PI and other study

personnel to the extent permitted by law. 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 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 monitor, applicable regulatory authorities (e.g., FDA), or other authorized representatives of the Sponsor may inspect all documents and records required to be maintained by the Site PI. This includes, but is not limited to, medical records (office, clinic, or hospital) and pharmacy records for the subjects in the trial. DEPRU will permit access to such records.

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.

14.6. Study Discontinuation

If the trial is discontinued, subjects who have signed the ICF and are administered study drug, will continue to be followed for safety for the duration of the scheduled follow-up period. No further study drug will be administered.

14.7. Costs, Subject Compensation, and Research Related Injuries

There is no cost to subjects for the research tests, procedures, and study drug while taking part in the trial. Procedures and treatment for clinical care may be billed to the subject, subject's insurance, or third party.

Subjects may be compensated for their participation in the trial. Compensation will be in accordance with the local IRB's policies and procedures, and subject to IRB approval.

14.8. Future Use of Stored Specimens and Data

Aside from plasma or BAL sample reanalysis, no future use of specimens is intended with this protocol. Plasma and BAL samples will be stored at –20 to –70°C for up to 189 days from collection to be used in case of need for reanalysis. All samples will remain coded to provide subject confidentiality. Samples will be discarded when analysis is complete or after 189 days, whichever is first.

15. DATA HANDLING AND RECORD KEEPING

The Site PI is responsible for ensuring the accuracy, completeness, legibility, and timeliness of reported data.

Data collection forms will be derived from the eCRF and provided by the DCC to record and maintain data for each subject enrolled in the trial. All data collection forms will be completed in a neat, legible manner to ensure accurate interpretation of data. Black or blue ink is required to ensure clarity of reproduced copies. When making a change or correction, 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 reported in the eCRF derived from the data collection forms should be consistent with the data collection forms or the discrepancies should be explained.

The Sponsor or designee will provide guidance to the Site PI and other study personnel on making corrections to the data collection forms and eCRF.

15.1. Data Management Responsibilities

All data collection forms and laboratory reports will be reviewed by the clinical team and data entry personnel, who will ensure that they are accurate and complete. AEs will be recorded on the appropriate data collection form, assessed for severity and relationship, and reviewed by the Site PI or appropriate sub-investigator.

Data collection is the responsibility of the study personnel at DEPRU under the supervision of the Site PI. During the trial, the Site PI will maintain complete and accurate documentation for the trial.

The DCC for the trial will be responsible for data management, quality review, analysis, and reporting.

15.2. Data Capture Methods

Clinical (including, but not limited to, AE/SAEs, concomitant medications, medical history, physical assessments, and clinical laboratory values), will be entered into a 21 CFR 11-compliant Internet Data Entry System provided by the DCC. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the data collection forms completed by the study personnel.

15.3. Types of Data

A variety of data will be collected and used in the aforementioned analysis. These data include, but are not limited to demographics, medical history and prior medications, hematology, coagulation, urinalysis and blood chemistry, vital signs, ECGs, and PK blood and BAL samples (Appendix A).

15.4. Timing/Reports

As the duration of the trial is short, an initial review of data is anticipated for QC purposes. All data will be thoroughly reviewed upon completion before analysis and writing of the study report.

15.5. Study Records Retention

DEPRU will maintain appropriate medical and research records for the trial, in compliance with ICH E6 R1, Section 4.9, regulatory and institutional requirements for the protection of confidentiality of subjects. DEPRU will permit authorized representatives of the Sponsor, DMID, and regulatory agencies to examine (and when required by applicable law, copy) clinical records for the purposes of clinical site monitoring, quality assurance reviews, audits, and evaluation of the study safety and progress. A Site Master File will be maintained to include essential documents.

Study documents will be retained for at least 2 years after the last marketing application approval or 2 years from the formal discontinuation of clinical development of an investigational drug. These documents will be retained for a longer period, if required by local regulations. No record will be destroyed without the written consent of the Sponsor.

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 Site PI, or DEPRU personnel. If deviations occur, corrective actions will be promptly developed and implemented by DEPRU.

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 and personnel 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 per the DCC protocol deviation reporting procedures.

All protocol deviations, as defined above, will be addressed in study subject data collection forms. A completed copy of the DMID Protocol Deviation Form will be maintained in the Regulatory File, as well as in the subject's chart. Protocol deviations will be sent to the local IRB/IEC per their guidelines. The Site PI and personnel are responsible for knowing and adhering to their IRB requirements.

16. PUBLICATION POLICY

Following completion of the trial, the investigator will publish the results of this research in a scientific journal. All manuscripts resulting from the trial will be reviewed by representatives from DEPRU, DMID, and Nabriva Therapeutics, Inc. Each institution will have at least 30 days to review the publication before submission.

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 Investigators will register the trial and post results.

The responsible party does not plan to request certification of delayed posting.

Refer to:

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

17. LITERATURE REFERENCES

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18. SUPPLEMENTS/APPENDICES

APPENDIX A: SCHEDULE OF STUDY PROCEDURES AND EVALUATIONS

Table 9: Schedule of study procedures and evaluations

Activities	Screening Visit	Baseline Visit	Treatment Phase	Discharge Visit	Telephone Follow-Up	Early Termination	Unscheduled Visit
	Day -30 to -1	Day -1 to 1	Days 1 to 2	Day 2	Day 3		
Informed Consent	X						
Review Eligibility Criteria	X	X					
Randomization		X					
Demographics	X						
Admission to DEPRU		X					
Medical History	X	X[a]					
Counsel on Birth Control	X			**X**		**X**	
Counsel on non-prescription medications	X						
Prior/Concomitant Medication Review	X	X	X	X	X	X	X
Collect and Record AEs/SAEs[b]			X	X	X	X	X
Height / BMI	X					X	
Vital Signs[c]	X	X	X	X		X	X
Physical Exam (including weight)	X	X		X		X	X[d]

Alcohol and Tobacco History[e]	X						
ECG (12-lead)[f]	X	X	X				X
HIV Antibody, Hepatitis B Ag, Hepatitis C Antibody	X						
Hem-Coag-UA[g]	X		X	X		X	
Chemistry[g]	X	X	X	X		X	
Lipids, LDH[g]	X		X	X		X	
Pregnancy Tests[h]	X	X					
Urine & Breath Tox Screens[i]	X	X					
SARS-CoV- 2 (COVID-19) Test[j]	X	X					
Place Two Peripheral IVs		X					
Study Drug Administration[k]			X				
Blood for Urea Assay[l]			X				
Blood PK Samples[m]			X				
Bronchoscopy and BAL[n]			X				
Discharge from DEPRU				X			

[a] Update medical history as appropriate since screening including non-pharmacologic treatments/procedures within 72 hours before enrollment in the eCRF

[b] Collect and record AEs and SAEs from the time the start of treatment (Day 1) through Day 3.

[c] Vital signs (heart rate, blood pressure, temperature, respiratory rate, and peripheral oxygen saturation), measured with subject in the supine or semi-recumbent position, will be obtained at the following timepoints: Screening, Baseline (Day -1), immediately prior (within 10 minutes) to each of three doses of Study Drug, at approximately 0.5, 1, 2, and 5 hours (each ± 10 minutes) as after the start of infusion for each dose, and before discharge from DEPRU. If vital signs are abnormal, repeated readings will be obtained up to twice more, 5 to 10 minutes apart. If they remain abnormal, unscheduled vital signs will be repeatedly obtained until they normalize (at investigator's discretion). Since changes in vital signs are expected during bronchoscopy, when a vital sign timepoint is scheduled during a subject's bronchoscopy, that time point will be measured immediately before the start of bronchoscopy.

[d] At unscheduled visits, weight may be performed as part of the physical exam but is not required

[e] Alcohol history within past 6 months, lifetime tobacco history

[f] ECGs will be performed in the supine or semi-recumbent position (after rest for at least 10 min) at 3 timepoints: at Screening, Day 1 (before first dose of Study Drug), and within 1-2 hours of the last dose (3rd dose) of study drug. In case of premature discontinuation of study drug, ECG is to be performed before discharge.

[g] At Screening, and before discharge (Day 2), obtain hematology labs (white blood cell count, hemoglobin, hematocrit, and platelet count, neutrophils, lymphocytes, monocytes, eosinophils, basophils); coagulation tests (aPTT and PT), chemistry labs (albumin, glucose, blood urea nitrogen (BUN), potassium, magnesium, calcium, sodium, phosphorus, total protein, creatinine, triglycerides, total cholesterol, CPK, phosphorus, AST, ALT, total bilirubin, direct bilirubin, ALP, LDH), and urinalysis (leukocyte esterase, blood, glucose, protein, pH, and specific gravity via dipstick with addition of urine microscopy if dipstick abnormal). At Baseline (Day -1 to Day 1) obtain albumin, glucose, BUN, potassium, magnesium, calcium, sodium, phosphorus, total protein, creatinine, CPK, AST, ALT, total bilirubin, and ALP. Approximately two hours prior to bronchoscopy (Day 2) obtain sodium, potassium, magnesium, calcium, and phosphorus.

[h] Serum pregnancy test for women of childbearing potential at screening, and urine pregnancy test for women of childbearing potential at Baseline (Day -1 to Day 1)

[i] Alcohol Breathalyzer test, Urine cotinine, and Urine drug screen at Screening and Baseline (Day -1 to Day 1/admission to DEPRU). Cotinine will be reported qualitatively as positive/negative. Urine drug screen will consist of: barbiturates, benzodiazepines, THC, cocaine, opiates, and amphetamine/methamphetamine.

[j.] Test for SARS-CoV-2 (COVID-19) at Screening or Baseline (Day -1 to Day 1) within 72 hours of admission

[k] Subjects will receive a total of three doses of ZTI-01 (fosfomycin disodium) 6g, administered every 8 hours, as 1-hour intravenous infusion (+10 minute window)

[l] Blood sample to determine plasma urea concentration will be obtained just before or during the scheduled bronchoscopy

[m] PK blood samples will be collected at 15 timepoints: before (within 10 minutes) the first, second, and third doses of study drug, at 30 minutes (during infusion), 1 hour (within 5 minutes after the end of infusion), 1 hour 15 minutes, 2, and 5 hours from the start time of the first dose of ZTI-01, and at 30 minutes (during infusion), 1 hour (within 5 minutes after the end of infusion), 1 hour 15 minutes, 2, 5, 8, and 12 hours from the start time of the third (last) infusion of ZTI-01. Blood PK samples will be drawn for all subjects within the following windows: -1 to -10 minutes for scheduled samples at pre-dose; +/- 5 minutes for scheduled samples at 30 minutes, 1 hour 15 minutes, and 2 hours; +0-5 minutes

after end of infusion for the scheduled sample at 1 hour; +/- 15 minutes for scheduled samples at 5, 8, and 12 hours. Actual times for dosing and sample will be recorded.

[n] Bronchoscopy with BAL at 30 minutes (during the 1-hr infusion), 1 hr 15 minutes, 2, 5, or 8 hours from T0 (defined as the start time of the last [third] infusion of ZTI-01). BAL PK samples will be obtained within +/- 15 minutes of scheduled timepoint. Actual times for dosing and sample will be recorded.

APPENDIX B: ADVERSE EVENT TOXICITY GRADING SCALE

ABBREVIATIONS: Abbreviations utilized in the Table:

ULN = Upper Limit of Normal	LLN = Lower Limit of Normal
R_x = Therapy	Req = Required
Mod = Moderate	IV = Intravenous
ADL = Activities of Daily Living	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 and do not interfere with the subject's daily activities.
GRADE 2	Moderate	Events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events 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. Severe events are usually incapacitating.

SERIOUS OR LIFE-THREATENING AEs

ANY clinical event that meets the SAE criteria, is considered a grade 3 event. (examples of clinical events considered to be life-threatening include, but are not limited to: seizures, coma, tetany, diabetic ketoacidosis, disseminated intravascular coagulation, diffuse petechiae, paralysis, acute psychosis, 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 participants in DMID trials.
- For parameters not included in the following Toxicity Tables, DEPRU should refer to the "Guide for Estimating Severity Grade" located above.
- Criteria are generally grouped by body system.
- Some protocols may have additional protocol specific grading criteria, which will supersede the use of these tables for specified criteria.

Table 10: Clinical adverse event definition and grading scale

Clinical Adverse Events				
VITAL SIGNS	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	
Fever (°C) *	38.0 – 38.4	38.5 – 38.9	≥39.0	* Oral temperature; no recent hot or cold beverages or smoking.
(°F) *	100.4 – 101.1	101.2 – 102.0	≥102.1	
Tachycardia - beats per minute	101 – 115 and >25% change from baseline	116 – 130 and >25% change from baseline	> 130 or ventricular dysrhythmias and >25% change from baseline	

Bradycardia - beats per minute	50 – 54 or 45 - 49 bpm if baseline <60 bpm - Subject is asymptomatic; no treatment required	45 – 49 or 40 - 44 if baseline <60 bpm - Subject is asymptomatic; no treatment required; Site PI/sub-investigator may confirm with ECG or 12-lead Holter monitors to rule out arrhythmia or advanced heart block.	< 45 or <40 bpm if baseline <60 bpm - Subject is symptomatic and/or requires treatment; Site PI/sub-investigator should confirm with ECG or 12-lead Holter monitors to rule out arrhythmia or advanced heart block	
Hypertension (systolic)- mm Hg	141-150	151-160	> 160	Assuming supine position, 10 min at rest conditions, not sleeping subjects measurements on the same arm and several concordant results.
Hypertension (diastolic) - mm Hg	91-95	96-100	> 100	
Hypotension (systolic) - mm Hg	85-89	80-84	< 80	
Tachypnea – breaths per minute	23-25	26-30	>30	
CARDIOVASCULAR	Grade 1	Grade 2	Grade 3	

Hemorrhage, Blood Loss	Estimated blood loss ≤ 100 mL	Estimated blood loss > 100 mL, no transfusion required	Transfusion required	
QTc interval (prolonged)	QTc 450-480 msec	QTc 481-500 msec	QTc >500 msec	
PR Interval (prolonged)	PR 200-250 msec and >25% change from baseline	PR >250 msec and >25% change from baseline	AV block 2nd degree Type II or higher OR ventricular pause >3 sec	
QRS Interval (prolonged)	QRS 120-150 msec and >25% change from baseline	QRS 150-180 msec and >25% change from baseline	New bundle branch block	
RESPIRATORY	Grade 1	Grade 2	Grade 3	
Cough	Transient- no treatment	Persistent cough;	Interferes with daily activities	
Bronchospasm, Acute	Transient; no treatment; 71% - 80% FEV1 of peak flow	Requires treatment; normalizes with bronchodilator; FEV1 60% - 70% (of peak flow)	No normalization with bronchodilator; FEV1 <60% of peak flow	
Dyspnea	Does not interfere with usual and social activities	Interferes with usual and social activities, no treatment	Prevents daily and usual social activity or requires treatment	
GASTROINTESTINAL	Grade 1	Grade 2	Grade 3	
Nausea	No interference with activity	Some interference with activity	Prevents daily activities	

Vomiting	No interference with activity or 1 - 2 episodes/24 hours	Some interference with activity or > 2 episodes/24 hours	Prevents daily activity or requires IV hydration	
Diarrhea	2 - 3 loose or watery stools or < 400 gm/24 hours	4 - 5 loose or watery stools or 400 - 800 gm/24 hours	6 or more loose or watery stools or > 800gms/24 hours or requires IV hydration	
REACTOGENICITY				
LOCAL REACTIONS	Grade 1	Grade 2	Grade 3	
Pain	Does not interfere with activity	Repeated use of non-narcotic pain reliever > 24 hours or interferes with activity	Any use of narcotic pain reliever or prevents daily activity	
Tenderness	Discomfort only to touch	Discomfort with movement	Significant discomfort at rest	
Erythema/Redness **	2.5 - 5 cm	5.1 - 10 cm	> 10 cm	** In addition to grading the measured local reaction at the greatest single diameter, the measurement should be recorded as a continuous variable.
Induration/Swelling ***	2.5 - 5 cm and does not interfere with activity	5.1 - 10 cm or interferes with activity	> 10 cm or prevents daily activity	***Induration/Swelling should be evaluated and graded using the functional scale as well as the

				actual measurement
SYSTEMIC	Grade 1	Grade 2	Grade 3	
Allergic Reaction	pruritus without rash	localized urticaria	generalized urticaria; angioedema or anaphylaxis	
Headache	No interference with activity	Repeated use of non-narcotic pain reliever > 24 hours or some interference with activity	Significant; any use of narcotic pain reliever or prevents daily activity	
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	
ALL OTHER CONDITIONS	Grade 1	Grade 2	Grade 3	
Illness or clinical adverse event (as defined according to applicable regulations)	No interference with activity	Some interference with activity not requiring medical intervention	Prevents daily activity and requires medical intervention	

Table 11: Laboratory adverse event definition and grading scale

LABORATORY ADVERSE EVENTS			
BLOOD, SERUM, PLASMA	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Sodium – Hyponatremia mEq/L	132 – <LLN	130 – <132	<130
Sodium – Hypernatremia mEq/L	>ULN – 148	>148 – 150	>150
Potassium – Hyperkalemia mEq/L	>ULN – 5.2	>5.2 - 5.4	>5.4
Potassium – Hypokalemia mEq/L	3.1-<LLN	3.0-<3.1	<3.0
Glucose – Hypoglycemia mg/dL	65 - 69	55 - <65	<55
Glucose – Hyperglycemia Fasting – mg/dL	>ULN - 120	>120 - 130	>130
Glucose – Hyperglycemia Random – mg/dL	141 - 159	>159 – 200	>200
Blood Urea Nitrogen mg/dL	21-26	>26 – 31	> 31
Creatinine – mg/dL	>ULN – 1.7	>1.7 – 2.0	>2.0
Calcium – hypocalcemia mg/dL	8.0 – <LLN	7.5 – <8.0	<7.5
Calcium – hypercalcemia mg/dL	>ULN – 11.0	>11.0 – 11.5	>11.5
Magnesium – hypomagnesemia mg/dL	1.3 – 1.7	1.1 – <1.3	<1.1
Phosphorous – hypophosphatemia mg/dL	2.1 – 2.2	1.9 – <2.1	<1.9
CPK – mg/dL	221-1000	>1000-1500	>1500
Albumin – Hypoalbuminemia g/dL	2.8 – 3.4	2.5 – <2.8	< 2.5
Total Protein – Hypoproteinemia g/dL	5.2 – <LLN	5.0 – <5.2	< 5.0
Alkaline phosphatase (ALP) – U/L	111 - 240	>240– 360	>360
AST U/L	42 – 105	>105-175	>175
ALT U/L (Female)	>ULN - 105	>105-175	>175
ALT U/L (Male)	>ULN – 105	>105-175	>175
Bilirubin (serum total) - mg/dL	1.6 - 2.5	>2.5 - 3.0	> 3.0
Direct bilirubin – mg/dL	0.7-2.0	>2.0-3.5	>3.5
Total cholesterol – mg/dL	301 – 400	>400-500	>500
Triglycerides – mg/dL	>500 – 750	>750 – 1000	>1000
Lactate dehydrogenase – U/L	>200-500	>500-800	>800
Hemoglobin (Female) - g/dL	11.0 – 11.9	9.5 – <11.0	< 9.5

Hemoglobin (Male) - g/dL	12.0 – 13.6	10.0 – <12.0	<10.0
WBC Increase - cell/mm3	9900 – 15,000	>15,000 – 20,000	> 20,000
WBC Decrease - cell/mm3	2,500 – 3100	1,500 – <2,500	< 1500
Lymphocytes Decrease - cell/mm3	500 – <600	400 – <500	< 400
Neutrophils Decrease - cell/mm3	1,500 - < 2000	1000 - <1500	< 1000
Eosinophils - cell/mm3	> 700 - 750	>750 - 1500	> 1500
Platelets Decreased - cell/mm3	120,000 – < 149,000	100,000 – <120,000	<100,000
PT – seconds (prothrombin time)	> ULN – 14.4	>14.4 – 15.7	> 15.7
aPTT – seconds (activated partial thromboplastin time)	> ULN – 42.1	>42.1 – 50.0	> 50.0
URINE	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Protein	1+	2+	>2+
Glucose	1+	2+	>2+
Blood (microscopic) - red blood cells per high power field (rbc/hpf)	5-10*	>10-50*	> 50 and/or gross blood*

* Isolated presence of blood in urinalysis is acceptable for menstruating females.

APPENDIX C: VENIPUNCTURE SCHEDULE AND VOLUMES

Table 12: Venipuncture schedule and volumes

Scheduled Laboratory Assessments	Study Days			Blood Volume per Sample (mL)	Total Number of Samples	Total Blood Volume (mL)
	Screening (Day -30 to Day -1)	Baseline (Day -1 to Day 1)	Treatment and Discharge (Days 1-2)			
Chemistry	X	X	X	9	3	27
Electrolytes (only)			X	4.5	1	4.5
Hematology	X		X	4	2	8
Coagulation	X		X	2.7	2	5.4
Serum Pregnancy	X			5	1	5
Serology[a]	X			8.5	1	8.5
Urea Blood Sample			X	4	1	4
PK Blood Samples			X	4	15	60
Total blood volume (mL)[b]	29.2	9	84.2			**122.4**

[a] Serology is for testing of HIV antibody, Hepatitis B antigen, Hepatitis C antibody.

[b] The total blood volume of 122.4 ml will be collected from each subject; blood volume per study phase is reflected at the bottom of each column.