A PHASE I, RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED, DOSE ESCALATION STUDY TO EVALUATE THE SAFETY, TOLERABILITY, AND PHARMACOKINETICS OF INTRAVENOUS ERTAPENEM IN COMBINATION WITH ZIDEBACTAM (WCK 6777) IN HEALTHY ADULT SUBJECTS

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STATEMENT OF COMPLIANCE

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

- United States (U.S.) Code of Federal Regulations (CFR) applicable to clinical trials (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 54, 21 CFR Part 56, and 21 CFR Part 312).
- International Council for Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use E6(R2) Good Clinical Practice (ICH E6 GCP): Integrated Addendum to ICH E6(R1) Guidance for Industry, published in the Federal Register (83 Federal Register 8882 (2018)), including the latest finalized National Institutes of Health (NIH) Clinical Terms of Award, as applicable.

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

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

SIGNATURE PAGE

The signature below constitutes the approval of the protocol and its attachments and provides the necessary assurances that the trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements as well as applicable U.S. federal regulations and ICH guidelines.

Site Investigator: Martin Kankam, MD, PhD, MPH, FAPCR

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Signature: _____

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LIST OF ABBREVIATIONS

AE	Adverse Event
Ae, urine	Amount of unchanged drug excreted in urine during each collection interval
Ae,urine(0-24)	Cumulative amount of unchanged drug excreted in urine from zero (predose) to 24 hours
Ae,urine(0-36)	Cumulative amount of unchanged drug excreted in urine from zero (predose) to 36 hours
ALT	Alanine Aminotransferase
AP	Alkaline Phosphatase
APTT	Activated Partial Thromboplastin Time
AST	Aspartate Aminotransferase
AUC	Area Under the Concentration – Time Curve
$AUC_{(0-t)}$	Area under the concentration - time curve from time zero to time t
$AUC_{(0-24)}$	Area under the concentration – time curve from time zero to 24 h
$AUC_{(0-\infty)}$	Area under the concentration - time curve from time zero to infinity
$AUC_{(0-last)}$	Area under the concentration - time curve from time zero to the last concentration above the lower limit of quantitation
$AUC_{(0-tau)}$	Area under the concentration – time curve to the end of the dosing interval
β-HCG	Beta Human Chorionic Gonadotropin
BL	β-lactamase
BLI	β-lactamase inhibitor
BMI	Body Mass Index
BP	Blood Pressure
bpm	Beats per Minute
BUN	Blood Urea Nitrogen
CAP	Community-acquired Pneumonia
CBC	Complete Blood Count
CDC	Centers for Disease Control and Prevention
CFR	Code of Federal Regulations
CHEM	Chemistry Panel
CI	Confidence Interval

cIAI	Complicated Intra-abdominal Infections
C_{avg}	Calculated average concentration during the dosing interval
C_{max}	Maximum Concentration
C_{min}	Minimum Concentration
CL_{CR}	Creatinine Clearance
CLr(0-24)	Renal clearance to 24 h after initial dose
CLr, ss	Renal clearance following multiple dosing
CL_T	Total Clearance
CMS	Clinical Materials Services
ConMed(s)	Concomitant Medication(s)
COAG	Coagulation
CPM	Clinical Project Manager
CRE	Carbapenem-Resistant Enterobacterales
CROMS	Clinical Research Operations and Management Support
CSR	Clinical Study Report
CTU	Clinical Trial Unit
cUTI	Complicated Urinary Tract Infection
DBP	Diastolic Blood Pressure
DVC	Dynport Vaccine Company, LLC, a GDIT company
dL	Deciliter
DMID	Division of Microbiology and Infectious Diseases, NIAID, NIH
eCRF	Electronic Case Report Form
EC	Ethics Committee
ECG	Electrocardiogram
EDC	Electronic Data Capture
ERT	Ertapenem
ESBL	Extended-Spectrum β-lactamases
ET	Early Termination
fe,urine(0-24)	Fraction of dose excreted unchanged in urine from zero (predose) to 24 hours

fe,urine(0-36)	Fraction of dose excreted unchanged in urine from zero (predose) to 36 hours
FEP	Cefepime
FDA	Food and Drug Administration
FH	Family History
FSH	Follicle-Stimulating Hormone
FWA	Federalwide Assurance
g	Gram(s)
GCP	Good Clinical Practice
GFR	Glomerular Filtration Rate
eGFR	Estimated GFR
h	Hour(s)
HBsAg	Hepatitis B Surface Antigen
Hct	Hematocrit
HCV	Hepatitis C Virus
HEENT	Head, Eyes, Ears, Nose, and Throat
HEM	Hematology Panel
Hgb	Hemoglobin
HIV	Human Immunodeficiency Virus
HLGT	High Level Group Term
HPF	High-Powered Field
HR	Heart Rate
IATA	International Air Transport Association
IB	Investigator's Brochure
IC_{50}	Half Maximal Inhibitory Concentration
ICF	Informed Consent Form
ICH	International Council for Harmonization
IMP	Imipenemase
IND	Investigational New Drug
INR	International Normalized Ratio
IRB	Institutional Review Board

IUD	Intrauterine Device
IV	Intravenous(ly)
kg	Kilogram(s)
LC-MS/MS	Liquid Chromatography with Tandem Mass Spectrometry
LLN	Lower Limit of Normal
MAD	Multiple Ascending Dose
MBL	Metallo-β-lactamases
MDR	Multiple Drug Resistant
MedDRA®	Medical Dictionary for Regulatory Activities
µg	Microgram(s)
mg	Milligram(s)
MH	Medical History
MIC	Minimum Inhibitory Concentration
min	Minute(s)
mL	Milliliter(s)
MM	Medical Monitor
mmHg	Millimeters of Mercury
mmol	Millimole(s)
MO	Medical Officer
MOP	Manual of Procedures
msec	Milliseconds(s)
N	Number (typically refers to the total number of subjects)
n	Number (typically refers to a subset of the total number of subjects)
NIAID	National Institute of Allergy and Infectious Diseases, NIH
NIH	National Institutes of Health
NOAEL	No Observable Adverse Effect Level
NSAID	Nonsteroidal Anti-Inflammatory Drug
OAT	Organic Anion Transporter
OPAT	Out-Patient Parenteral Antibiotic Therapy
OER	Office of Extramural Research, NIH
OTC	Over the Counter

PD	Pharmacodynamic(s)
PE	Physical Examination
PI	Principal Investigator
PK	Pharmacokinetic(s)
POC	Point of Contact
Protime	Prothrombin time
PVG	Pharmacovigilance Group
QID	Four times a day
QTc	Corrected QT Interval of the ECG
QTcF	Corrected QT interval of the ECG using Fridericia's Formula
RAUC	Accumulation ratio for AUC
RBC	Red Blood Cell
RC_{AUC}	Accumulation ratio for AUC
RC_{max}	Accumulation ratio for C_{max}
RP	Research Pharmacist
RR	Respiratory Rate
SAD	Single Ascending Dose
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SBP	Systolic Blood Pressure
SD	Standard Deviation
SDCC	Statistical and Data Coordinating Center
sec	Second(s)
SMC	Safety Monitoring Committee
SOC	System Organ Class
T	Temperature
$t_{1/2}$	Terminal Elimination Half-life
T_{max}	Time of Maximum Concentration
T_{min}	Time of Minimum Concentration
TEAE	Treatment-emergent Adverse Event
TID	Three times a day

TLF	Tables, Listing and Figures
UA	Urinalysis
ULN	Upper Limit of Normal
US	United States
Vd	Volume of Distribution
VS	Vital Signs
Vd,ss	Volume of distribution at steady state
WBC	White Blood Cell
WCK 5107	Zidebactam
WCK 5222	Combination of Cefepime with Zidebactam
WCK 6777	Combination of Ertapenem with Zidebactam
WHO	World Health Organization
ZID	Zidebactam

PROTOCOL SUMMARY

Title:	A Phase I, Randomized, Double-blind, Placebo-controlled, Dose Escalation Study to Evaluate the Safety, Tolerability, and Pharmacokinetics of Intravenous Ertapenem in Combination with Zidebactam (WCK 6777) in Healthy Adult Subjects
Phase:	1
Population:	Approximately 52 evaluable healthy male or female subjects 18 to 45 years of age (both inclusive) at the time of dosing
Number of Sites:	One (Altasciences Clinical Kansas, Inc.)
Study Duration:	Approximately 11 months from study activation to last subject last visit
Subject Participation Duration:	Each subject will participate in the study for up to 42 days. The trial will consist of a Screening Period of up to 27 days as outpatient (Day -28 to Day -2); a Check-in/Baseline/Enrollment Day (Day -1); a Treatment Period of minimum of 7 nights/8 days (Days 1 to 8) as inpatient, with daily dosing on Days 1 to 7 and discharge on Day 8; and an out-patient Follow-up Period of 3 to 6 days (Days 9 to 14) with one site visit on Day 11 (+3 days) (Final Visit).
Description of Agents:	WCK 6777 is the designation for the combined preparation containing Ertapenem (ERT) and Zidebactam (ZID; WCK 5107) in equal amounts, ranging from 2 g (1 g of each compound) to 6 g (3 g of each compound). ERT is an approved antibacterial drug that belongs to the class of carbapenem antibiotics. ERT is provided as lyophilized powder of ERT sodium in a glass vial containing ERT 1 g/vial. The doses to be used range from 1 g to 3 g per injection daily for 7 days, depending on the study cohort. Sterile Water for Injection, USP will be used to reconstitute the drug and sterile 0.9% Sodium Chloride, USP to further dilute the reconstituted solution to the desired volume for IV infusion, which is 100 mL for the ERT 1-g combination with 1-g ZID, as the 2-g WCK 6777 (1-g ERT + 1-g ZID), and 250 mL either

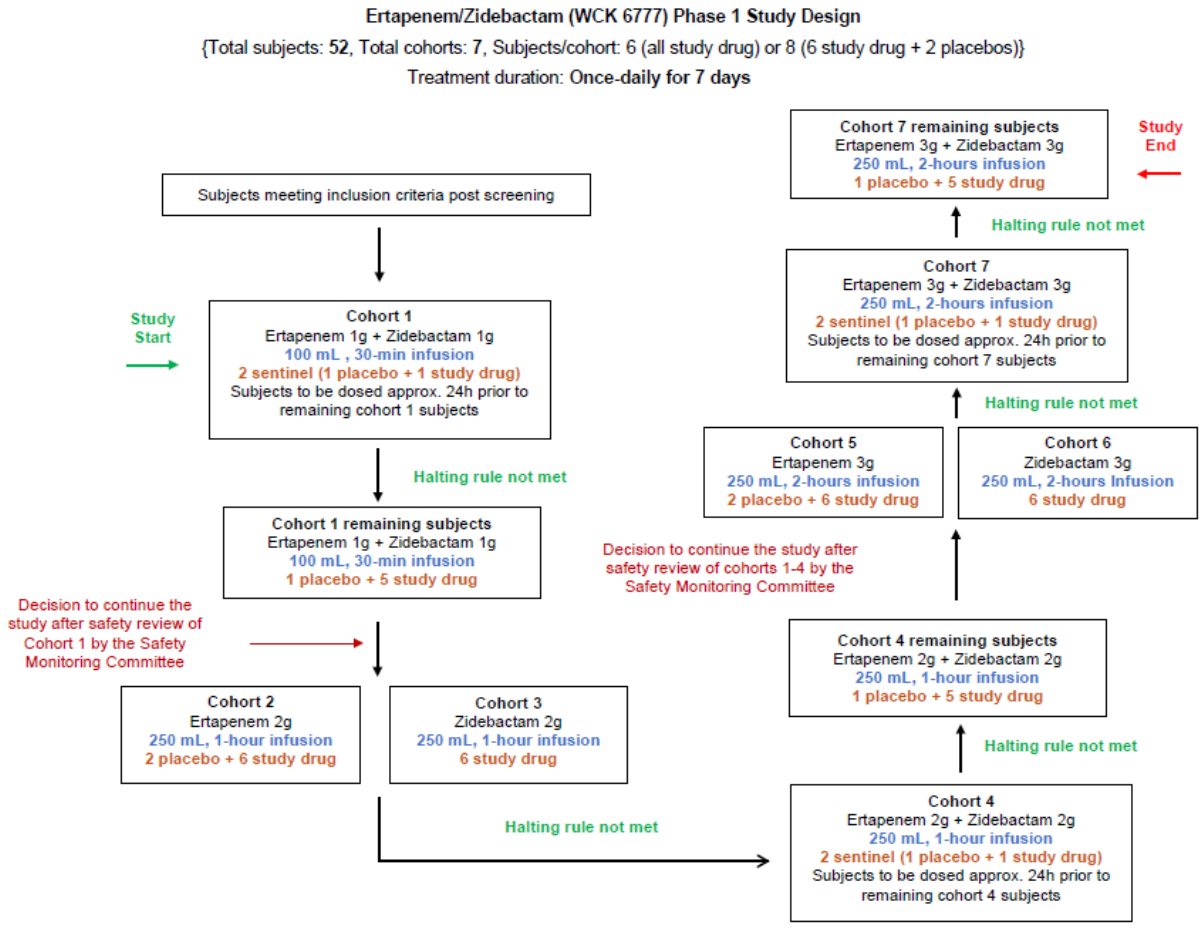
	for the 2-g or 3-g standalone ERT or for the combination with 2-g or 3-g ZID, respectively as the 4-g WCK 6777 (2-g ERT + 2-g ZID) or the 6-g WCK 6777 (3-g ERT + 3-g ZID). ERT will be administered by IV infusion over a period from 30 min to 2 hours, depending on the drug amount delivered following study design. ZID is a synthetic compound that belongs to a novel class of bicyclic diazabicyclooctane (DBO) with dual functions as β-lactamase inhibitor and β-lactam enhancer. It is under investigation as an antibacterial agent in combination with cefepime (WCK 5222). ZID dihydrate is provided in lyophilized powder in a glass vial containing 1 g/vial. The doses to be used range from 1 g to 3 g per infusion daily for 7 days depending on the study cohort. Sterile Water for Injection, USP will be used to reconstitute the drug and sterile 0.9% Sodium Chloride, USP to further dilute it to the desired volume for IV infusion, which is 100 mL for the ZID 1-g combination with ERT, as the 2 g WCK 6777 (1 g ZID + 1 g ERT), and 250 mL either for the 2 g or 3 g standalone ZID or for the combination with 2 g or 3 g ERT, respectively for the 4 g WCK 6777 (2 g ZID + 2 g ERT) or the 6 g WCK 6777 (3 g ZID + 3 g ERT). ZID will be administered by IV infusion over a period from 30 min to 2 h, depending on the drug amount delivered following study design.
Objectives:	**Primary** Assess the safety and tolerability of three dose-escalating regimens of WCK 6777 (ERT and ZID combination) and two-dose escalating regimens of standalone ERT or ZID following single daily doses for 7 days in healthy adult subjects **Secondary** Characterize the PK profiles in plasma (total & free) and in urine of three dose-escalating regimens of WCK 6777 (ERT and ZID combination) and two dose-escalating regimens of standalone ERT or ZID following a single initial dose on Day 1 and after single daily doses for 7 days in healthy adult subjects
Outcome Measures:	**Primary** Type, incidence, severity, and relatedness to study drug of all treatment-emergent adverse events (AEs) and serious adverse events

	(SAEs) in each treatment cohort from the first dose through the follow-up period **Secondary** PK parameter estimates and calculated exposure measures in plasma (total and free) and urine for ERT and ZID, when given alone and in combination following a single initial dose on Day 1 and after single daily dose administration for 7 days
Description of Study Design:	This is a Phase 1, single center study to investigate the safety, tolerability, and pharmacokinetics (PK) of three dose-level groups of WCK 6777 (ERT and ZID combination), and two dose-level groups of ERT alone and ZID (WCK 5107) alone in 52 healthy adult male and female subjects aged 18 to 45 years old (both inclusive). Seven treatment cohorts will be evaluated in this study (Table 1 and Figure 1). WCK 6777 will be evaluated in three cohorts - Cohorts 1, 4 and 7- of 8 subjects each (6 study drug combinations and 2 placebos); ERT will be evaluated alone in two cohorts – Cohorts 2 and 5- of 8 subject each (6 ERT and 2 placebos); and ZID will be evaluated in two cohorts, Cohorts 3 and 6, of 6 subjects each (all ZID). The study will be placebo-controlled and double-blinded in all cohorts except Cohorts 3 and 6. No placebo subjects are included in standalone ZID cohorts, since adequate safety data for higher doses of ZID alone in comparison with placebo are available from completed Phase 1 studies of WCK 5107 (ZID) alone and the ZID-only arms of WCK 5222 (cefepime + ZID) studies. In each cohort, either study drugs alone or their combination will be administered by a single intravenous infusion (IV) of 100 mL daily for 7 consecutive days in Cohort 1 or 250 mL daily for 7 consecutive days in Cohorts 2 to 7. For each treatment cohort, however, the dose will be progressively escalated from 1 g/daily to 2 g/daily and to 3 g/daily, and the duration of infusion time increased from 30 min to 1 h and to 2 h over the course of the study. In Cohort 1, WCK 6777 2 g (ERT 1 g/daily combined with ZID 1 g/daily) will be administered in 30 (±5) minutes (min); in Cohort 2 (ERT 2 g/daily), Cohort 3 (ZID 2 g/daily), and Cohort 4 (WCK 6777 4 g [ERT 2 g/daily combined with ZID 2 g/daily]) the study drug(s) will be administered in 60 (±10) min, and in Cohort 5 (ERT 3 g/daily),

	Cohort 6 (ZID 3 g daily) and Cohort 7 (WCK 6777 6 g [ERT 3 g/daily combined with ZID 3 g/daily]), the study drug(s) will be administered in 120 (±10) min. (See Table 1). Study enrollment will proceed from Cohort 1 to Cohort 7 starting with the lowest dose and escalating to the next higher dose. Individual dose Cohorts 2 and 3 and 5 and 6 will run in parallel. For each combination treatment cohort (Cohorts 1, 4 and 7), two sentinel subjects will receive study treatments (one WCK 6777 and one placebo; drug/ placebo ratio 1:1) and followed for safety for approximately 24 h prior to dosing of the remaining 6 subjects in the cohort. If no halting rules have been met 24 hours after start of dosing, dosing of sentinel subjects will continue for the remainder of the study and the additional 6 subjects in the cohort will be dosed (5 will receive WCK 6777 and 1 will receive placebo; drug/placebo ratio 5:1). Sentinels will not be employed in the single treatment cohorts. Blood (plasma) and urine samples will be collected for measuring drug concentrations for PK analysis for 24 h after starting the first IV infusion on Day 1, and for 24 h after starting the last IV infusion on Day 7. Plasma concentrations of study drugs will also be measured before and 12 h after dosing on Days 2 to 6. Total and free drug concentrations of ERT and ZID will be measured in plasma and total drug concentration of ERT and ZID in the urine by validated LC-MS/MS assays. Safety data will be monitored from the time of infusion on Day 1 to the last visit (Day 11+3 days) and will consist of: daily assessments of treatment-emergent AEs, vital signs and symptom-directed physical examination (PE); clinical laboratory safety tests (Day -1, Days 2, 4, 6 and Day 8 (prior to the discharge from the study site]), and 12-lead ECGs, and a complete PE on the last visit (Day 11 +3 days). AEs will be assessed by the study clinicians for severity or seriousness, and relatedness to study drug(s) and include outcome and duration. The decision for dose escalation from combination Cohort 1 (1 g ERT + 1 g ZID) to the next planned higher single dose Cohorts 2 (2 g ERT) and 3 (2 g ZID), and from combination Cohort 4 (2 g ERT

	+ 2 g ZID) to single dose Cohorts 5 (3 g ERT) and 6 (3 g ERT) will be based on unblinded review of cumulative interim safety data to Day 11 (+3 days) of each combination cohort by the Safety Monitoring Committee (SMC). The recommendation to continue to combination Cohort 4 and to combination Cohort 7 (3 g ERT + 3 g ZID) will be made by the study team (DMID Medical Officer [MO] and Medical Monitor [MM] and Site PI) after review of blinded safety data in Cohorts 2 and 3 and Cohorts 5 and 6, respectively, to determine if halting rules are met. The SMC will review all interim safety data, not only those that meet pre-specified halting rules. after completion of Cohort 1 and Cohorts 1-4, and all final safety data after database lock. If no halting criteria are met, the decision to continue dose escalation will be made and announced by DMID. If halting criteria are met, an *ad hoc* meeting of the SMC will convene to review the unblinded data and make recommendations about dose escalation and the continuation of the trial. The SMC will also review the aggregate data in the first 4 cohorts, before dosing the remaining cohorts, and the cumulative safety data at the end of the trial after the database lock.
Estimated Time to Complete Enrollment	Up to 10 months (from dosing of first subject to final visit of last subject).

Figure 1 Description Schematic of Study Design



1 KEY ROLES

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2 BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 Background Information

Antibiotics were one of the significant medical discoveries in the 20th century and they save 200,000 American lives annually, extend life expectancy, and facilitate major advances in medicine and surgery [1]. Despite best efforts, our ability to use antibiotics safely and effectively has been compromised by the emergence of antibiotic resistance. Antimicrobial resistance is an ongoing public-health problem of critical importance and is considered as one of the top ten threats to global health by the World Health Organization (WHO) [2]. Among the various emerging resistant bacterial pathogens, carbapenem-resistant bacteria are particularly worrisome given that carbapenems have long been considered the most reliable last-resort treatment for infections caused by Gram-negative pathogens. Carbapenem resistance predominately manifests in various Gram-negative organisms including Enterobacterales, which have been recognized as an urgent threat by both the CDC and WHO that require aggressive action [3, 4]. While a variety of mechanisms contribute to carbapenem resistance among Enterobacterales, it is driven in a large part by the increasing prevalence and complexity of a variety of β-lactamases. The most important of these are the class A serine carbapenemases, such as KPC, class B metallo-β-lactamases (MBLs), such as Verona integron Metallo β-lactamase (VIM), imipenemase (IMP) and New Delhi Metallo β-lactamase (NDM) and class D carbapenemases, such as OXA-48 types [5].

Evidence shows that the only way to stay ahead of the resistance curve is to follow the best infection control practices, use antibiotics prudently, and bring new agents to the market [6]. There has been an impressive antibiotic drug development response in recent years to combat the rise in antibiotic resistant pathogens and several intravenous (IV) antibiotics with activity against certain carbapenem- resistant Enterobacterales (CREs) encountered in clinical practice have been approved by both the FDA and EMA [7, 8]. While these recently approved agents are welcomed additions to our antibiotic armamentarium, nearly all are required to be administered by IV, multiple times-a-day with infusion times ranging from 1 to 3 hours. The dosing and administration requirements of these newer agents are associated with some logistical challenges, but they are largely manageable in hospitalized patients. However, infections due to CREs are of substantial concern across all clinical settings, including the community setting, and none of newer agents with enhanced CRE activity are conducive for outpatient use. Plazomicin is the only recently approved agent against CREs that is dosed once daily but it lacks comprehensive coverage of CREs (such as strains producing NDM due to co-expression of 16S rRNA methyl transferases) and does not possess a safety profile commensurate for outpatient parenteral

antimicrobial therapies (OPAT) [9]. Among epidemiologic studies that identified the occurrence of community-onset CRE infections, the prevalence ranged from 8 to 30% globally and from 5 to 11% in the United States [10].

The rise in community-onset CRE infections highlights the critical need for effective OPAT [11]. OPAT has the potential to be the 'standard-of-care' for the management of many infections requiring IV antimicrobial therapy like CRE infections. For some conditions, initiation of OPAT in the outpatient or emergency department setting may allow avoidance of hospitalization entirely [12]. Avoidable hospitalizations impose a significant burden on already stressed health-care systems which could be mitigated to a large extent if an effective OPAT is available. The availability of an effective OPAT also facilitates early discharge of clinically stable patients. Data shows that patients often remain in the hospital after their acute infection resolves, and it is possible to discharge these patients with OPAT without compromising the clinical outcomes [13]. Beyond the potential cost benefits, early discharge of suitable patients with OPAT has been shown to improve patient satisfaction, reduce healthcare-associated infections, and allow resources to be reallocated to increase patient care throughput. A potential OPAT solution for patients with CRE infections in development is the combination of ERT with ZID (WCK 6777, ERT-ZID), which will be evaluated in this study as a once-daily intravenous infusion (IV) administered over 0.5 to 2 hours.

2.2 Ertapenem and Zidebactam (WCK 6777)

WCK 6777 is the code name for Wockhardt's proprietary combination of ertapenem (ERT) and zidebactam (ZID).

ERT is a once-daily IV carbapenem antibiotic that is FDA-approved for multiple indications in the United States. It is a well-established OPAT drug with robust activity against infections due to extended-spectrum β-lactamase (ESBL) producing-Enterobacterales [14]. However, ERT has no activity against CRE. Moreover, strains harboring multiple ESBLs and AmpC and decreased expression of porins also tend to show reduced susceptibility to ERT owing to hampered uptake of ERT [15]. ZID is a novel diazabicyclooctane (DBO) [16]. In addition to β-lactamase inhibition like other DBOs, such as avibactam and relebactam, ZID forms a strong covalent interaction with the penicillin binding protein 2 (PBP2) present in all the clinically important, carbapenem-resistant Gram-negative pathogens [17-19]. This feature, termed as β-lactam enhancer action, is maximized when ZID is combined with β-lactams like ERT and cefepime that bind to PBPs other than PBP2. The antibacterial effects of the PBP-optimized ZID/β-lactam combinations are (1) enhanced microbiologic activity against MBL-expressing Gram-negative bacteria albeit ZID lacks β-lactamase inhibitory activity against MBLs [20] and (2) substantial reductions in %*f* T>MIC requirements for the partner β-lactam [21-22]. Both these advantages

were demonstrated *in vitro* and *in vivo* with ZID combined with cefepime (FEP) (WCK 5222, Wockhardt's three times a day [TID] drug) [21, 23]. WCK 5222 is active against Gram-negative organisms expressing all four Ambler classes of β- lactamases and has pharmacokinetic/ pharmacodynamics (PK/PD) susceptibility breakpoint of 64 µg/mL as compared to 8 µg/mL for cefepime alone [23-26]. Presently, cefepime/ZID is pending evaluation in Phase 3 trials for complicated urinary tract infections (cUTI) and ventilated-nosocomial pneumonia.

This proposed Phase 1 healthy subject study is aimed to evaluate the safety, tolerability, and pharmacokinetics (PK) of the once-daily IV combination of ERT with ZID (WCK 6777). To date, pre-clinical animal PK/PD studies that simulated clinical exposure profiles of ERT 2 g and ZID 2 g, IV once- daily demonstrated bactericidal activity against KPC-, OXA-48, and MBL-producing Enterobacterales with ERT/ZID MIC values up to 8 µg/mL [27]. Although the FDA-approved dosing regimen for ERT in human is 1 g IV daily [28], the safety of IV ERT doses up to 3 g once-daily have been evaluated in Phase 1 and 2 studies [29]. Clinical studies with ZID have established safety for a single highest dose of 3 g and total divided daily dose of 6 g/day [30, 31], with some clinical studies characterizing the PK of ZID in pulmonary tissues of healthy subjects [32] as well as in plasma of subjects with impaired renal function [33] (ClinicalTrials.gov Identifiers: NCT02532140, NCT02674347, NCT02707107, NCT03630094, NCT02942810, NCT03554304). However, no human safety and PK data are available with these agents when they are used in combination as a once-daily dosing regimen. Therefore, the proposed Phase 1 studies will assess the safety, tolerability, and PK of escalating regimens of ERT in combination with ZID to determine its suitability for continued clinical development.

2.2.1 Zidebactam (WCK 5107)

2.2.1.1 Physicochemical properties

Zidebactam dihydrate (CAS No. 1996664-59-5) is a proprietary New Chemical Entity (NCE) of Wockhardt coded as WCK 5107. It is a bicyclo-acyl-hydrazide (DBO pharmacophore) chemically identified as (2S, 5R)-7-Oxo-6-sulphooxy-2-[N'-((R)-piperidin-3-carbonyl)-hydrazinocarbonyl]-1, 6-diaza-bicyclo [3.2.1] octane dihydrate. The structure and absolute stereochemistry of ZID has been confirmed though various instrumental methods of analysis such as elemental analysis, NMR spectrometry, mass spectrometry, and single crystal X-ray analysis. ZID has a molecular formula of $C_{13}H_{21}N_5O_7S.2H_2O$ and a molecular weight of 427.45 daltons (includes two water molecules). ZID exists as pale yellow to white crystalline powder. It is slightly hygroscopic and is freely soluble in water and insoluble in dichloromethane [34].

2.2.1.2 Mechanism of Action

ZID has a novel, dual mechanism of action, involving broad-spectrum inhibition of various serine β-lactamases including Class A, C and certain Class D β-lactamases, and as β-lactam enhancer due to selective, high-affinity binding (IC_{50} 0.01 to 0.26 μM) to Gram-negative PBP2, (a key enzyme involved in cell wall synthesis and maintenance of the rod shape), of in all the four clinically significant Gram-negative pathogens, namely, *E. coli*, *K. pneumoniae*, *P. aeruginosa* and *A. baumannii* [17-19, 34].

2.2.1.3 Antibacterial activity

By virtue of strong affinity towards Gram-negative PBP2 and also pan-β-lactamase stability, ZID exhibits antibacterial activity against certain pathogens on standalone basis. For example, ZID $MIC_{50/90}$ for Enterobacterales and *P. aeruginosa* were 0.12/>64 μg/mL and 0.5/>64 μg/mL, respectively. It should be noted that though standalone ZID shows impressive *in vitro* activity, it does not translate into adequate *in vivo* efficacy due to single PBP2 binding action [20, 34].

2.2.1.4 *In Vitro* Drug Interaction Studies

- **CYP450 Studies:** ZID up to a 500 μM (equivalent to 195.7 μg/mL) did not induce CYP1A2 or CYP3A4 enzyme activity and mRNA levels. Hence ZID is unlikely to cause CYP mediated drug-drug interactions (DDI) [34].

  ZID is not an inhibitor of all key human CYP450 isoforms (CYP1A2, CYP2C8, and CYP2C9 CYP3A4) up to 2,000 μM (782.5 μg/mL) and it did not inhibit CYP2C19, and CYP2D6 up to tested concentration of 300 μM (117.4 μg/mL) [34].

- **Transporter Studies:** ZID is unlikely to cause clinical DDIs involving the human transporters due to non-inhibitory potential towards P-gp, BCRP, OATP1B1, OATP1B3, OAT1, OAT3, and OCT2 human transporters. Additionally, ZID does not have inhibitory activity towards human transporters like MRP4, OCT1 and BSEP [34].

  ZID substrate potential for renal transporters (OAT1, OAT2, OCT2, MRP4) was studied *in vitro* both in the presence and absence of ERT. ZID did not show substrate potential for OAT1, OAT2, OCT2, or MRP4 [34].

2.2.1.5 Genetic Toxicity

Zidebactam was evaluated through a battery of *in-vitro* (bacterial reverse mutation assay and chromosomal aberration in human lymphocytes) and *in-vivo* (mouse micronucleus test) genotoxicity tests. In mouse micronucleus test, the exposure in terms of AUC at NOAEL dose (1302 mg/kg) was 1194.5 to 1441.2 mg·h/L. This exposure is about 3.5 to 4 times of clinical exposure obtained at the intended clinical dose of Zidebactam 2 g daily in WCK 6777. Zidebactam did not show clastogenic potential in both *in-vitro* and *in-vivo* genotoxicity studies.

Further studies are needed to assess the risk of mutagenicity. Thus, considering the available data and shorter therapeutic duration envisaged of up to 14 days, the risk of carcinogenicity is low. [34].

2.2.1.6 Preclinical *In Vivo* Safety Profile

In preclinical safety assessment, ZID was found to be safe for vital organ systems based on their extensive evaluation in safety pharmacology studies for cardiovascular, gastrointestinal, respiratory and central nervous systems. The IV No Observed Adverse Effect Level (NOAEL) doses, based on a 28-day repeat dose toxicity study in rat and dog are 800 and 750 mg/kg/day, respectively and the corresponding exposure in terms of 24-hour (h) area under the plasma drug concentration -time curves (AUCs) are 1950 and 1962 mg·h/L, respectively. On the basis of ZID NOAEL dose exposures in dog (750 mg/kg/day) and rat (800 mg/kg/day) and anticipated ZID clinical exposure of 360 mg·h/L at 2 g/day, a safety window of approximately 5.4 times is envisaged [34].

In a 13-week repeat-dose intravenous toxicity study of ZID in Beagle dogs, the NOAEL and Low Observed Adverse Effect Level (LOAEL) were 250 and 500 mg/kg, respectively. Based on the body surface area, these doses in Beagle dog correspond to per day administration of 9.7 g and 19.4 g of ZID for a 70 kg human, respectively [34].

2.2.2 Ertapenem

2.2.2.1 Physicochemical Properties

ERT is generally available as ertapenem sodium (CAS No. 153773-82-1), and chemically identified as [4R-[3(3S*,5S*),4α,5β,6β(R*)]]-3-[[5-[[(3-carboxyphenyl)amino] carbonyl]-3-pyrrolidinyl]thio]-6-(1-hydroxyethyl)-4-methyl-7-oxo-1-azabicyclo[3.2.0] hept-2-ene-2-carboxylic acid monosodium salt. It has a molecular formula $C_{22}H_{24}N_3O_7SNa$ and a molecular weight of 497.50 daltons. ERT sodium is a white to off-white hygroscopic, weakly crystalline powder. It is soluble in water and 0.9% sodium chloride solution, practically insoluble in ethanol, and insoluble in isopropyl acetate and tetrahydrofuran [28].

2.2.2.2 Mechanism of Action

ERT rapidly binds to PBPs of Enterobacterales with greater affinity towards PBP3 and PBP2. Such a rapid rate of PBP binding of ERT is an enabling feature as it would be able to induce elongation even in the presence of inactivating enzymes. In a *Pseudomonas* PBP binding study, ERT showed high-affinity binding to all the essential PBPs, such as PBP2, PBP3 and PBP1a/b in *P. aeruginosa* [34].

2.2.2.3 Antibacterial Activity

ERT is a broad-spectrum, carbapenem antibiotic with coverage spectrum encompassing Gram-negative (including ESBL-expressing strains), Gram-positive and anaerobic pathogens. ERT has potent *in vitro* activity against β-lactamase-expressing Enterobacterales including those that produce class A ESBLs and class C enzymes. ERT is not effective against Enterobacterales expressing ERT-hydrolyzing β-lactamases, such as KPC, MBL and Class D- OXA-carbapenemases. ERT also has limited activity against *Pseudomonas aeruginosa* owing to its vulnerability to pseudomonal efflux and poor uptake [34].

2.2.2.4 *In Vitro* Drug Interaction Studies

- **CYP450 Studies**: *In vitro* studies in human liver microsomes indicate that ERT does not inhibit metabolism mediated by any of the following cytochrome p450 (CYP) isoforms: 1A2, 2C9, 2C19, 2D6, 2E1 and 3A4 [34].

- **Transporter Studies:** *In vitro* studies have established that ERT does not inhibit P-glycoprotein mediated efflux nor it is a substrate of the same efflux pump [21].

  ERT in presence and absence of ZID was assessed for its renal transporter substrate potential against OAT1, OAT3, OCT2 and MRP4 transporters at higher concentration. The results of transporter substrate study indicate that ertapenem is a substrate of OAT3 transporter; it is likely to be a substrate of OAT1 and MRP4 and non-substrate of OCT2 transporter. The substrate activity of ERT remained unaffected in presence of ZID (1000 μM) [34].

2.2.2.5 Genetic Toxicity

No long-term studies in animals have been performed to evaluate the carcinogenic potential of ertapenem. Ertapenem was not genotoxic in *in vitro* or *in vivo* assays, including: an alkaline elution/rat hepatocyte assay, a chromosomal aberration assay in Chinese hamster ovary cells, a TK6 human lymphoblastoid cell mutagenesis assay and a mouse micronucleus cells, a TK6 human lymphoblastoid cell mutagenesis assay and a mouse micronucleus assay [28, 34].

2.2.2.6 Preclinical and Clinical Safety Profile

The safety of ERT is well established through a range of preclinical studies in rodents and non-rodents and clinical studies. ERT was approved for marketing in 2001 [28]. Safety and tolerability of ERT even at doses higher than the currently approved 1 g/day has been well established in clinical studies conducted by Merck. In Phase 1, the maximum dose of ERT studied was 3 g/day with no serious adverse events (AEs). In Phase 2 trials, higher ERT dose of

1.5 g and 2 g were also evaluated; for which the pattern and frequency of AEs were similar to those who received 1 g of ERT [29, 34].

2.2.2.7 Clinical Indication

In the U.S., ERT is indicated as a once-a-day parenteral carbapenem antibiotic in adults and pediatric patients (3 months of age and older) for the treatment of the following moderate to severe infections caused by susceptible bacteria: complicated urinary tract infections (cUTI) including pyelonephritis, community-acquired pneumonia (CAP), complicated intra-abdominal infection (cIAI) and complicated skin and skin-structure infections including diabetic foot infections without osteomyelitis, acute pelvic infections including postpartum endomyometritis, septic abortion and post-surgical gynecologic infections. ERT is also indicated in adults for the prophylaxis of surgical site infection following elective colorectal surgery [28, 34].

2.2.3 WCK 6777 (Combination of ERT and ZID)

WCK 6777 is a combination of equal amounts of ERT, a carbapenem antibiotic, and ZID, a non-β-lactam entity which acts as a β-lactam enhancer. It is being developed as once-a day parenteral therapy for the treatment of Gram-negative infections. The indications described for ERT would be targeted for WCK 6777 in clinical settings.

2.2.3.1 Mechanism of Action

In Gram-negatives, WCK 6777 operates on the basis of β-lactam enhancer mechanism of action. ZID binds to Gram-negative PBP2 and ERT being a potent PBP3 and PBP2 binding agent (additional binding to PBP1a/b in Enterobacterales), the combination exerts bactericidal action against MDR pathogens through complementary binding of multiple PBPs. This mechanism of action obviates the need of β-lactamase inhibition leading to the activity against pathogens expressing ZID non-inhibitable β-lactamases, such as MBLs. ERT-ZID combination is further benefitted by ZID's ability to inhibit several class A (including KPC) and class C β-lactamases [34].

2.2.3.2 Antibacterial activity – *In Vitro*

The $MIC_{50/90}$ of ERT-ZID against KPC-, OXA-48/181- and MBL-expressing Enterobacterales were 0.25/2, 1/2, and 0.5/4 µg/mL, respectively. For these set of organisms, the MIC_{90} of standalone ERT or ZID was >128 µg/mL. ERT-ZID combination was also active against *P. aeruginosa* with MIC_{50} of 4 µg/mL and MIC_{90} of 8 µg/mL. Time-kill studies demonstrated synergistic bactericidal action to the extent of 1 to 2 log_{10} kill upon 4-hour (h) exposure of ERT-ZID against wild type, ERT-resistant Enterobacterales and efflux hyper-expressing and OprD-down regulated *P. aeruginosa*.

Time-kill studies have shown that ERT's affinity to bind PBP3 in combination with PBP2 binding when combined with ZID, elicits potent synergistic bactericidality as a result of the inhibition of multiple essential PBPs. Thus, ZID enhances the pharmacodynamic action of ERT leading to activity against ERT-resistant MDR strains. With this augmented pharmacodynamic effect, the duration of the pharmacodynamic relevant exposures of ERT is significantly lowered, enhancing the therapeutic coverage of pathogens including those that express ERT-impacting resistance mechanisms. WCK 6777 also evokes a bactericidal response against *P. aeruginosa* as a result of PBP3 and PBP2 binding by ERT and ZID, respectively. Such PBP-mediated bactericidal action overcomes ERT-impacting resistance mechanisms such as efflux and ERT-hydrolysing β-lactamases that likely hamper the *in vitro* activity of ERT against *P. aeruginosa*. The inclusion of *P. aeruginosa* within the therapeutic scope of WCK 6777 would broaden its therapeutic utility as a step-down de-escalation therapy for several TID and QID combination products used for treatment of *P. aeruginosa* causing nosocomial infections [34].

2.2.3.3 Antibacterial activity – *In Vivo*

In vivo efficacy of ERT-ZID was assessed in a translational standard neutropenic mouse lung infection. ERT, ZID and ERT-ZID were tested at human-simulated regimens (HSR) in uranyl nitrate-treated, neutropenic mice which produced free plasma exposures of respective drugs mimicking the human free plasma exposures from once-daily administered 2 g of ERT and 2 g ZID. Against 24 carbapenem-resistant Enterobacterales which comprised of KPC or OXA-48-like or NDM-expressing strains, ERT or ZID HSR was ineffective while ERT-ZID HSR showed a consistent 1 to 2.5 $\log_{10}$ kill. A similar study was undertaken independently at Centre for Anti-infective Research and Development, Hartford Hospitals, wherein ERT/ZID HSR evoked >1 $\log_{10}$ CFU reduction (exceeding translational endpoint of 1 $\log_{10}$ kill) in 18/21 of KPC or OXA-48-like or NDM Enterobacterales and stasis to 0.5 $\log_{10}$ kill was observed in the remaining isolates [27, 34].

2.2.3.4 Safety Pharmacology – *In Vitro*

- **hERG inhibition assay** - At the highest tested concentration (1461 μM), there was a 10% inhibition of hERG current. The IC_{50} for ZID could be greater than 1461 μM (571.5 μg/mL). This concentration of ZID is 3.8-fold higher than the intended clinical C_{max} of ZID at dose of 2 g [34].

- **Receptor binding and enzyme assays** - ZID did not cause ≥ 50% inhibition of any of the enzymes or receptors at concentration of 500 μM (1.3-fold of clinical C_{max} of ZID 2 g), demonstrating lack of secondary pharmacodynamic targets [34].

2.2.3.5 Preclinical Safety – *In Vivo*

ERT-ZID up to the highest dose studied (1,200 + 1,000 mg/kg) in rats did not affect any of the respiratory functions or neurobehavioral parameters in a head-out plethysmography and a functional observational battery test, respectively. The maximum concentration (C_{max}) of ERT and ZID at these doses was about 3.9 and 5.3 times higher than the intended therapeutic C_{max} levels, respectively. Further, in a 28-day repeat dose toxicity study in rats, ERT-ZID (800 + 800 and 1600 + 1600 mg/kg/day) did not show any clinical signs of systemic toxicity. The no-observed-adverse-effect-level (NOAEL) of ERT-ZID for this study was adjudged as 1600+1600 mg/kg/day. The corresponding plasma AUC of ERT and ZID, 3700 and 4540 mg·h/L, were about 3.6 and 12.6 times higher the expected therapeutic exposures, respectively, indicating a broad safety margin. US FDA has granted a waiver for the 28-days study in non-rodent species in light of adequate data on preclinical and clinical safety available for both ERT and ZID. [34].

2.3 Clinical Experience

2.3.1 Human Studies with Zidebactam

ZID has been studied in Phase 1 human trial as WCK 5107 and is also being developed in combination with cefepime (FEP) as WCK 5222 (q8h) and is slated to enter Phase 3 clinical studies.

ZID has been administered to 91 healthy adult human volunteers in two Phase 1 double-blind, placebo-controlled single ascending dose (SAD) and multiple-ascending dose (MAD) studies conducted in the U.S. in healthy volunteers (W-5107-101; NCT02532140, and W-5107-102; NCT02674347). Doses ranged from 0.25 g to 3 g daily in the SAD study and 3 g (1 g q8 h) to 6 g (2 g q8 h) in the MAD study. In addition, 1 g (approximately 200 μCi) of [14C]-ZID has been administered to 8 healthy adult male volunteers in a Metabolism and Excretion study [30, 31, 34].

2.3.1.1 Safety and Tolerability

In the SAD study (W-5107-101), there were no deaths, serious adverse events (SAEs) or discontinuations due to AEs. No subjects on placebo reported any TEAEs. The majority of TEAEs were mild. One subject in the 1 g ZID cohort reported urticaria, and 1 subject in the 2 g cohort reported headache. In the cross-over cohorts, 5 subjects reported TEAEs that were considered treatment related, one with oral hypoesthesia (1 g dose ZID), 1 vulvovaginal mycotic infection (2 g FEP), 1 headache (1 g ZID+2 g FEP), 1 headache (2 g ZID+2 g FEP) and 1 vaginal discharge (2 g ZID+2 g FEP) [34].

In the MAD study (W-5107-102), there were no deaths, serious adverse events (SAEs) or discontinuations due to AEs. All TEAEs were mild in severity. Three subjects (37.5%) in Cohort 1 reported TEAEs, 2 due to the procedure (infusion site extravasation and 1 infusion site erythema), and 1 each myalgia, headache and vulvovaginal discomfort. In Cohort 2, there were 3 TEAEs of headache, and 2 of vessel puncture site hematoma. In the Placebo Cohort, 2 subjects reported headache, 2 infusion site erythema and 2 vessel puncture site pain. In the metabolism and excretion study (Q-5107-103), all AEs were mild in severity and were assessed as related to ZID. Overall, 3 subjects (37.5%) reported 5 TEAEs (3 AEs of diarrhea, 2 AEs of infrequent bowel movements) [34].

No deaths, serious adverse events (SAEs) or discontinuations due to AEs were reported during the study. No subjects on placebo reported any AEs [34].

Clinical safety of ZID is also supported through WCK 5222 (FEP-ZID) Phase 1 studies, in which 233 healthy volunteers were dosed with Zidebactam or cefepime/ZID, with ZID doses ranging from 3 g (1 g q8h) to 6 g (2 g q8h) daily for 7 to 10 days and FEP 6 g (2 g q8 h) daily. (ClinicalTrials.gov Identifiers: NCT02532140, NCT02707107, NCT03554304, NCT03630094, NCT02942810). These studies included a thorough QT study, which did not report QT prolongation or any cardiovascular AEs. No safety concerns were observed in any study. Both cefepime and ZID, at high doses, were well tolerated with a safety profile consistent with the β-lactam class [30-34].

2.3.1.2 Pharmacokinetics

ZID pharmacokinetics (PK), safety and tolerability have been investigated in Phase 1 clinical studies (W-5107-101 and W-5107-102). Intravenous ZID was assessed through single ascending dose (SAD) and multiple ascending dose (MAD) regimens in healthy adult (>18 years) human subjects. These randomized, double-blinded, placebo-controlled studies involved single escalating IV doses (0.25 to 3 g) and multiple IV doses (1 and 2 g, q8h for 7 days), administered as a 1 h infusion. In single ascending dose studies, ZID showed a linear PK; Geometric mean $AUC_{(0-inf)}$ ranged from 44.3 to 458 mg·h/L and C_{max} ranged from 16.5 to 174 µg/mL. ZID elimination half-life (1.8 to 2.4 h) was appeared to be independent of dose. The observed median T_{max} coincided with the respective cohort infusion duration within the 0.25 g to 3 g dose range. Renal clearance (4.85 L/h to 6.77 L/h) approximated the plasma clearance and appeared to be independent of the administered dose. In repeat dose study, exposure parameters were similar for each dosing interval within each dosing day. The plasma exposures of ZID were linear and comparable on Day 1 and Day 7. The majority (>80%) of the administered dose of ZID in the SAD study was excreted as unchanged drug within the first 0 to 6 h of the collection interval,

with the complete dose recovered within 24 h post-dose. In the MAD study, the majority of the administered q8h doses of ZID were excreted as unchanged drug within each dosing interval (0 to 8 h, 8 to 16 h, and 16 to 24 h collection intervals), with near complete dose recovery within 24 h post AM dosing on Day 1 (92% to 95%), and within 48 h post AM dosing on Day 7 (95% to 99%) [34].

In addition, 1 g (approximately 200 μCi) of [^{14}C]-ZID has been administered to 8 healthy adult male volunteers in a Metabolism and Excretion study conducted in U.S. In this study maximum plasma ZID concentrations were observed at the end of infusion for all subjects, after which plasma concentrations of ZID appeared to decline in a generally monophasic manner, with arithmetic mean $t_{1/2}$ of approximately 2 hours. The primary route of elimination of total radioactivity was in urine, with 94.6% of the dose recovered. Excretion of total radioactivity in feces was negligible (0.235%). An arithmetic mean of 102% of the administered dose was excreted as unchanged ZID in urine, with the majority being recovered within the first 12 hours after start of infusion [34].

2.3.2 Human Studies with Ertapenem

2.3.2.1 Safety and Tolerability

ERT is an antibiotic approved by the FDA for human use by prescription in 2001. Extensive safety data (mostly post-marketing) is available for standalone ERT, which should be taken into account for an ongoing safety assessment of the ERT-ZID combination in clinical studies, both of assessment of causality of adverse events, as well as expectedness of any Serious Adverse Events (SAE). Clinical trials enrolled 1,954 patients treated with ERT. Most adverse experiences reported in these clinical trials were described as mild to moderate in severity. ERT was discontinued due to adverse experiences in 4.7% of patients. The most common drug-related adverse experiences in patients treated with ERT were diarrhea (5.5%), infused vein complication (3.7%), nausea (3.1%), headache (2.2%), and vaginitis in females (2.1%). Merck-conducted clinical studies involving higher ertapenem doses of 1.5 g, 2 g or 3 g daily were administered to approximately 150 subjects [28, 29]. In these studies, ERT 2 g was well-tolerated with minimal adverse events (AEs) of diarrhea, nausea, vomiting, somnolence and headache of mild to moderate severity, and asymptomatic increases in hepatic transaminases and platelet counts that were reversible and not clinically significant. The infusion duration of the 3 g IV dose initially was 1 h, but this was changed to 2 h due to the apparent association of nausea with the more rapid infusion rate. In Phase 2 studies, the pattern and frequency of AEs in patients who received 1.5 g or 2 g of ERT were similar to that of 1 g ERT [28, 29, 34]. A complete list of TEAEs reported in clinical trials with ERT as well as post-marketing studies is available in the Prescribing Information for Ertapenem Sodium for Injection, and in the IB [28, 34, 37].

2.3.2.2 Pharmacokinetics

The single- and multiple-dose pharmacokinetics of ERT at doses up to 3 g were examined in healthy young men and women volunteers. Plasma and urine samples collected were analyzed using reversed-phase high-performance liquid chromatography with UV detection. ERT is highly bound to plasma protein. The protein binding changes from ~95% bound at concentrations of <50 μg/ml to ~92% bound at concentrations of 150 μg/ml (concentration at the end of a 30-min infusion following the 1-g dose). The nonlinear protein binding of ERT resulted in a slightly less than dose proportional increase in the area under the curve from 0 h to infinity (AUC0-∞) of total ERT. The single-dose AUC0-∞ of unbound ERT was nearly dose proportional over the dose range of 0.5 to 2 g. The mean concentration of ERT in plasma ranged from ~145 to 175 μg/mL at the end of a 30-min infusion, from ~30 to 34 μg/mL at 6 h, and from ~9 to 11 μg/mL at 12 h. The mean plasma $t_{1/2}$ ranged from 3.8 to 4.4 h. About 45% of the plasma clearance (CLp) was via renal clearance. The remainder of the CLp was primarily via the formation of the β-lactam ring-opened metabolite that was excreted in urine. There were no clinically significant differences between the pharmacokinetics of ERT in men and women. ERT does not accumulate after multiple once-daily dosing [35].

2.3.3 Human Studies with WCK 6777

WCK 6777 has not been studied in humans. This is the first in human study to investigate the safety and PK of ERT and ZID in combination.

2.4 Rationale

2.4.1 Study Rationale

The aim of the clinical development program for WCK 6777 is to allow a full assessment of safety and efficacy as well as ensuring that the product is rapidly made available to fulfill an unmet medical need in the treatment of infections due to multi-drug resistant (MDR) bacteria.

A Phase 1 study is required given that human safety and PK data are not available for ERT and ZID administered in combination. The following observations support conduct of this study: (a) Non-clinical mechanism of action (MOA) and PK/PD studies establish ERT/ZID 2 g/2 g QD as a potential treatment of patients with CRE infections; (b) Multiple Phase 1 studies conducted for ZID alone or in combination with FEP demonstrated safety and tolerability of ZID at multiple daily doses up to 6 g (2 g q8h) administered over 7 to 10 days; (c) Higher IV ERT doses of 2 g and 3 g QD have been studied in previous phase 1 and 2 studies (n = 101) [28,29]; (d) The preclinical toxicity studies of WCK 6777 in rats and dogs employing doses that generated

exposures higher than the clinical exposures reported for ERT 2 g and ZID 2 g suggest that the combination would be safe and tolerable.

2.4.2 Rationale for Dose Selection

In vivo PK/PD studies involving neutropenic mouse lung infection models have demonstrated that the ERT-ZID combination exerts significant bactericidal action against KPC, OXA-48-like and MBL-expressing Enterobacterales at mouse exposures that correspond to 2 g q24h unbound clinical exposures of ERT and 2 g q24h unbound exposures of ZID obtained when dosed individually [34].

The IV No Observed Adverse Effect Level (NOAEL) doses, based on a 28-day repeat dose toxicity study for ZID in rat and dog, are 800 and 750 mg/kg/day, respectively, and the corresponding exposure in terms of 24-hour (h) area under the plasma drug concentration-time curves (AUCs) are 1950 and 1962 µg·h/mL, respectively. On the basis of ZID NOAEL dose exposures in dog (750 mg/kg/day) and rat (800 mg/kg/day) and anticipated ZID clinical exposure of 360 µg·h/mL at 2 g/day, a safety window of approximately 5.4 times is estimated [34].

Extrapolation of the dog IV NOAEL dose of 750 mg/kg to a human dose (based on body surface area) would lead to a human equivalent dose for ZID of 416 mg/kg, corresponding to 29 g for a 70 kg human. Likewise, considering the NOAEL dose (800 mg/kg) in rat, the human equivalent dose for ZID would be 64 mg/kg, corresponding to 9 g for a 70 kg human [34].

2.5 Identified and Potential Risks and Benefits Associated with the Study

2.5.1 Risks with ZID

ZID alone has been administered to 91 healthy adult human volunteers in two Phase 1 studies [SAD and MAD] conducted in the U.S. In addition, 1 g (approximately 200 µCi) of [14C]-ZID has been administered to 8 healthy adult male volunteers in a Metabolism and Excretion study conducted in U.S.

Overall, there were no SAEs or AEs that led to discontinuation of participants. The most common AE reported was headache, and it was mild in the majority of subjects, with one subject reporting moderate headache. Infusion site erythema and infusion site pain have also been reported as side effects, both considered related to the placement of an IV catheter than a reaction associated with the study drug.

2.5.2 Risks with ERT

Extensive safety data (mostly post-marketing) is available for standalone ERT. The latter should be taken into account for an ongoing safety assessment of the ERT-ZID combination in clinical studies, both for the assessment of causality of adverse events, as well as expectedness of any SAEs.

Most adverse experiences reported in clinical studies with ERT, including those involving higher ertapenem doses of 1.5 g, 2 g or 3 g daily, were described as mild to moderate in severity. ERT was discontinued due to adverse experiences in 4.7% of patients. The most common drug-related adverse experiences in patients treated with ERT were diarrhea (5.5%), infused vein complication (3.7%), nausea (3.1%), headache (2.2%), and vaginitis in females (2.1%). The infusion duration of the 3 g IV dose initially was 1 h, but this was changed to 2 h due to the apparent association of nausea with the more rapid infusion rate. In Phase 2 studies, the pattern and frequency of AEs in patients who received 1.5 g or 2 g of ERT were similar to that of 1 g ERT [28, 29]. The safety profile of ERT is expected to be similar to that described during its clinical use when administered alone; however, as for all drug studies, there may be unexpected AEs with ERT that have not been observed to date.

2.5.3 Risks with WCK 6777

The ERT-ZID combination has not been tested in humans. Preclinical data suggest that there are no significant system organ effects and the doses to be used in the study are below NOAEL for both drugs in rats and dogs. The safety profile of WCK 6777 is expected to be similar to that described for each drug alone [34]; however, as for all drug studies, there may be unexpected AEs with WCK 6777 that have not been observed with ERT or ZID alone to date.

2.5.4 Venipuncture and IV Catheter Risks

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

IV catheter placement: An indwelling catheter may be placed in an arm vein (preferably antecubital) for frequent blood drawing for PK measurements on Days 1 to Day 8 and another one in a vein on the opposite arm for IV infusion of the study drugs. The catheter may cause phlebitis with signs of redness and warmth at or near the IV insertion site, and thrombophlebitis with a hard area palpable near the IV insertion site. These risks are minimal as the IV catheters, when used, are only used briefly after dosing. Careful inspection of the catheter site, including visualization of blood return, withdrawal of the catheter if needed, and change every 72 to 96 h

will minimize this risk. There is a risk of infection; however, this is a small risk as aseptic technique will be used.

2.5.5 Additional Risks

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

Blood draws: The amount of blood drawn is about 24 mL at Screening Visit, 15 mL on Day -1, 371 mL during the inpatient period (Days 1 to 8), 15 mL at Day 11 (last visit), and 425 mL during the entire trial. Additionally, small amounts of blood loss may occur if an IV catheter is used or additional blood samples are collected (for repeat laboratory testing, TEAE evaluation, etc.). Overall, the amount of blood that may be drawn during the trial is within the amount that are considered safe to be drawn during short or extended periods, respectively, and not excessive for the safety and PK assessment requirements of Phase 1 trials. However, there is a small risk that some subjects may develop mild symptoms of hypovolemia or anemia during the trial. These are reversible with specific treatments (e.g., fluid replacement, good nutrition, vitamins or iron supplementation).

2.6 Known Potential Benefits

The trial has no direct benefit for healthy participants. Knowledge gained in the trial could be of future benefit to public health and to individuals with infections, who might benefit if the study drug combination is licensed. The medical benefit would be to patients in the future from a potential new addition to the drugs available for the treatment of serious, antibiotic-resistant nosocomial and community acquired infections with increase efficacy and lower toxicity than available drug regiments. An additional benefit to future patients would be the ability to administer effective therapy for these infections in an out-patient setting, thus decreasing the need for hospitalization and associated costs.

Subjects will be promptly notified of any clinical test results that would have an impact on their health. Screening evaluations may detect previously unknown abnormalities that could be clinically significant. This could benefit a subject, as it may lead to earlier diagnostic evaluation and treatment of an underlying disorder.

2.7 Risk/Benefit Ratio

This ratio cannot be calculated by available data. Since high dose safety has been established for ERT and ZID individually, the risk for an SAE occurring in a subject treated with single or daily doses of each study drug separately in doses 1 to 3 g is considered to be very low. On the other hand, the risk of WCK 6777 (combined ERT and ZID) is not known. Preclinical studies have shown no potential DDIs through effects on enzymes and transporters. Selection of the

combined human doses by appropriate methods for estimating human exposures from animal studies, suggest that the maximum exposure in humans will be at levels several-fold lower than NOAELs in rats and dogs. Data collected in the preclinical and clinical development of ZID and ERT/ZID to date, support further clinical development. Subject selection is based on carefully considered eligibility criteria. Subjects will be treated in an inpatient unit and closely monitored during the trial, so that appropriate emergency care can be provided immediately if an acute event occurs. As an additional measure of risk mitigation, each WCK 6777 cohort involves sentinel dosing in two subjects and remaining subjects will be dosed if no halting rules have been met after 24 hours of dosing to sentinel subjects.

There is no medical benefit to healthy study volunteers from participation in this clinical trial.

3 OBJECTIVES AND OUTCOME MEASURES

3.1 Study Objectives

Primary

- Assess the safety and tolerability of three dose-escalating regimens of WCK 6777 (ERT and ZID combination) and two dose-escalating regimens of standalone ERT or ZID following single daily doses for 7 days in healthy adult subjects

Secondary

- Characterize the PK profiles in plasma (total and free) and in urine of three dose-escalating regimens of WCK 6777 (ERT and ZID combination) and the two dose-escalating regimens of standalone ERT or ZID following single daily doses for 7 days in healthy adult subjects

3.2 Study Outcome Measures

Primary

- Type, incidence, severity, and relatedness to study drug of all treatment-emergent adverse events AEs and serious adverse events (SAEs) in each treatment cohort from the first dose through the follow-up period

Secondary

- PK parameter estimates and calculated exposure measures in plasma (total and free) and urine for ERT and ZID, when given alone and in combination following a single initial dose on Day 1 and after single daily dose administration for 7 days

4 STUDY DESIGN

This is a Phase 1, single center study to investigate the safety, tolerability, and pharmacokinetics (PK) of three dose-escalating regimens of WCK 6777 (ERT+ZID), and two dose-escalating regimens of ERT alone and ZID (WCK 5107) alone in 52 healthy adult male and female subjects aged 18 to 45 years old.

Seven treatment cohorts will be evaluated in this study (Table 1). WCK 6777 (ERT+ZID) will be evaluated in three cohorts - Cohorts 1, 4 and 7 - of 8 subjects each (6 study drug combination and 2 placebos); ERT will be evaluated in two cohorts - Cohorts 2 and 5 - of 8 subject each (6 ERT and 2 placebo); and ZID will be evaluated in two cohorts - Cohorts 3 and 6 - of 6 subjects each (all 6 ZID). The study will be placebo-controlled and double-blinded in all cohorts except Cohorts 3 and 6. No placebo subjects are included in standalone ZID cohorts, since adequate safety data for higher doses of ZID are available [34].

In each cohort, ERT and ZID will be administered either alone or in combination by a single intravenous infusion (IV) of 100 mL daily for 7 consecutive days in Cohort 1 and 250 mL in Cohorts 2 to 7. The dose will be progressively escalated from 2 g to 3 g/daily for each study drug individually, and from 2 g to 4 g to 6 g for WCK 6777 [ERT+ZID]) and, depending on the cohort, the duration of infusion time will increase from 30 min to 1 h to 2 h over the course of the study. Thus, in Cohort 1, 2 g WCK 6777 (ERT 1 g combined with ZID 1 g), the infusion solution (100 mL) will be administered in 30 (±5) minutes; in Cohort 2 (ERT 2 g/daily), Cohort 3 (ZID 2 g/daily) and Cohort 4 (4 g WCK 6777; ERT 2 g combined with ZID 2 g), the infusion solution (250 mL) will be administered in 60 (±10) minutes; and in Cohort 5 (ERT 3 g/daily) , Cohort 6 (ZID 3 g daily) and Cohort 7 (6 g WCK 6777; ERT 3 g combined with ZID 3 g), the infusion solution (250 mL) will be administered in 120 (±10) minutes**.**

Table 1 Study Treatment Cohort

Cohort No.	Study Drug-Dose	Infusion time - frequency	Subjects
Cohort 1	WCK 6777 2 g (ERT 1 g + ZID 1 g) or placebo	30 (±5) minutes - once daily-for 7 days, 100 mL per dose	8 (6 drug combination, 2 placebo)
Cohort 2	ERT 2 g or placebo	60 (±10) minutes - once daily-for 7 days, 250 mL per dose	8 (6 drug, 2 placebo)
Cohort 3	ZID 2 g	60 (±10) minutes - once daily-for 7 days, 250 mL per dose	6 (all drug)
Cohort 4	WCK 6777 4 g (ERT 2 g + ZID 2 g) or placebo	60 (±10) minutes - once daily-for 7 days, 250 mL per dose	8 (6 drug combination, 2 placebo)
Cohort 5	ERT 3 g or placebo	120 (±10) minutes - once daily-for 7 days, 250 mL per dose	8 (6 drug, 2 placebo)

Table 1 Study Treatment Cohort

Cohort No.	Study Drug-Dose	Infusion time - frequency	Subjects
Cohort 6	ZID 3 g	120 (±10) minutes - once daily-for 7 days, 250 mL per dose	6 (all drug)
Cohort 7	WCK 6777 6 g (ERT 3 g + ZID 3 g) or placebo	120 (±10) minutes - once daily-for 7 days, 250 mL per dose	8 (6 drug combination, 2 placebo)

The treatment regimen for the first combination cohort (Cohort 1), WCK 6777 2 g (ERT 1 g with ZID 1 g daily; 30-minute infusion), was selected because there are no human safety and PK data available with these agents in combination. The second combination cohort (Cohort 4) will evaluate the safety and PK of the anticipated therapeutic combination regimen of WCK 6777 4 g (ERT 2 g with ZID 2 g daily; 1-h infusion). The third combination-treatment cohort (Cohort 7) will evaluate the safety and PK of higher daily doses of WCK 6777 6 g (ERT 3 g + ZID 3 g daily; 2-h infusion). The primary purpose of the ERT 3 g with ZID 3 g combination treatment cohort is to ensure maximal flexibility in the clinical development program in the event there is a need to evaluate more intensive combination daily dosing regimens and different infusion strategies in future clinical trials. Moreover, 3 + 3 g/daily combination regimen will provide critical information on dose proportionality, PK linearity, and plasma protein binding linearity. As it is well-established that healthy subjects do not fully reflect the real-world inter-subject variability observed in patients [36], the higher daily dosing regimen will also provide data on the safety, tolerability, and PK of upper range exposures likely to be encountered in clinical practice with ERT 2 g + ZID 2 g. To mitigate the risk of higher osmolality-linked infusion reactions, the volume of each infusion in the 2-g and 3-g dose standalone and combined groups (Cohorts 2-7) will be increased to 250 mL. The duration of infusions in the 2-g and 3-g cohorts were chosen to mitigate infusion duration-related AEs (nausea, vomiting) with ERT doses >2 g [29]. In four of the seven cohorts (Cohorts 2, 3, 5 and 6), single 2 g and 3 g ERT or ZID infusions will be administered. ERT 1 g daily and ZID 1 g daily will not be evaluated as single drug treatment cohorts as there are sufficient safety, tolerability, and PK data with these standalone regimens [28, 29, 34].

In all cohorts, 6 subjects will receive study drug product. Two additional subjects will receive placebo in the 2 g/daily and 3 g/daily ERT single treatment cohorts and in ERT-ZID combination cohorts to maximize interpretation of the safety and tolerability of the observed results. The single drug treatment cohorts are being conducted to collect the baseline safety and pharmacokinetic data. Specifically, the single-treatment arms of ERT and ZID are necessary to assess for the presence of any PK interactions between the two constituents and to deduce the role of each, in the event of any safety or tolerability signals in the combination cohorts. In conjunction with the escalating dose combination regimens, the single treatment drug cohorts

will also provide information on dose-PK proportionality, which is necessary for building robust individual population PK models for ERT and ZID, which, when supplemented with PK/PD studies, will help justify the dose regimen for advanced clinical trials.

Study enrollment will proceed from Cohort 1 to the last Cohort 7 starting with the lowest dose and escalating to the next higher dose. Enrollment of single drug Cohorts 2 and 3, and 5 and 6 will be conducted in parallel and completed before enrollment of combination Cohorts 4 and 7, respectively. Placebo subjects will be enrolled in WCK 6777 cohorts (Cohorts 1, 4 and 7) and in the ERT alone cohorts (Cohorts 2 and 5), but not in the ZID cohorts (Cohorts 3 and 6). Sentinel subjects will be enrolled in the WCK 6777 cohorts but not in the ERT and ZID standalone cohorts. For each WCK 6777 cohort (Cohorts 1, 4 and 7), two sentinel subjects will receive study drug product on the first day of treatment, one active drug combination and the other placebo (normal saline) (active drug/placebo ratio, 1:1) and followed for approximately 24 hours. If no halting rules are met after 24 hours of dosing, dosing of sentinel subjects will continue for the remainder of the study, and the additional 6 subjects in the cohort will be dosed (5 will receive the study drug combination and 1 will receive placebo (active/placebo ratio, 5:1). Alternate subjects may be admitted in all cohorts at check-in and, if they are not used on the following dosing day, they may be used on another dosing day in the same cohort or in a future cohort if they continue to meet eligibility criteria.

Blood (plasma) and urine samples will be collected for measuring drug concentrations of ERT and ZID and for PK analysis in all cohorts before dosing, for 24 h after starting the first IV infusion on Day 1, and for 24 h after starting the last IV infusion on Day 7. In addition, blood (plasma) will be collected before dosing and 12 hours after dosing on Days 2 to 6 for the measurement of concentrations of ERT and ZID. Total and free drug concentration will be measured in plasma and total drug concentration in the urine for ERT and ZID by validated LC-MS/MS assays. The plasma and urine PK profiles for each study drug in the single dose ZID and ERT cohorts and the WCK 6777 cohorts will be analyzed by non-compartmental methods (NCA).

Safety data will be monitored from the time of infusion on Day 1 to the last visit, Day 11 (+3 days), and changes will be compared to the pre-dosing baseline (Day -1). Evaluations will consist of: assessments of TEAEs (Day 1 to Day 11), symptom-directed (focused) physical examination (PE) as needed (Days 1 to 7) and complete PE (Days 8 and 11); vital signs (Day 1 to 8, Day 11); Clinical laboratory safety tests (Days 2, 4, 6, 8 and 11); and 12-lead ECGs (Days 8 and 11). AEs will be assessed by study clinicians for severity or seriousness and relatedness and will include outcome and duration.

Dose escalation from combination Cohort 1 (1 g ERT + 1 g ZID) to higher single dose Cohorts 2 (2 g ERT) and 3 (2 g ZID), and from combination Cohort 4 (2 g ERT + 2 g ZID) to higher single dose Cohorts 5 (3 g ERT) and 6 (3 g ZID) will be based on the SMC review of unblinded cumulative interim safety data collected through study Day 11 (+3 days) in each combination

treatment cohort. (See Section 9.5.3). The recommendation to continue to the next planned combination cohorts (i.e., Cohort 4 and Cohort 7 [3 g ERT + 3 g ZID]), will be made by the study team (DMID MO and MM and the site PI) after review of blinded safety data in Cohorts 2 and 3 and Cohorts 5 and 6, respectively. The SDCC will provide a report of number of subjects meeting dose escalation halting criteria. The decision to continue dose escalation to the next single or combination dose cohorts will be made by DMID after review of the SMC or the study team recommendation. If the decision is to continue dose escalation, the next in line cohort(s) will enroll.

If halting criteria are met, an *ad hoc* meeting of the Safety Monitoring Committee (SMC) will convene to review blinded data (or unblinded in a closed meeting) and make recommendation about dose escalation (See Section 9.5.1). The SMC will also convene in *ad hoc* meetings per DMID request to review a potential safety concern identified by either the Site PI or the DMID MM.

Finally, in scheduled meetings, the SMC will review unblinded aggregate data in the first 4 cohorts, before dosing will continue in the remaining cohorts (Cohorts 5, 6 and 7), and cumulative safety data at the end of the trial after database lock (See Section 9.6.1).

4.1 Sub-studies

No sub-studies are planned.

5 STUDY ENROLLMENT AND WITHDRAWAL

Only subjects who meet all inclusion criteria and no exclusion criteria will be eligible for enrollment into the trial.

5.1 Subject Inclusion Criteria

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

1. Provide a signed and dated written informed consent and agrees to comply with the study procedures and length of confinement to the research site.
2. Be able to understand and willing to comply with study procedures, restrictions, and requirements, as determined by the Site Principal Investigator (PI) or authorized clinician(s) (listed on FDA Form 1572).
3. Adults 18 to 45 years of age inclusive, including non-pregnant, non-lactating females.
4. Have suitable veins for cannulation or repeated venipuncture.
5. Be in good general health at the time of enrollment.
   - **Note 1**: Determined by medical history (MH), medication use, physical examination (PE), vital signs (VS), clinical laboratory tests including estimated creatinine clearance (CL_{CR}) ≥80 mL/min by the Cockcroft-Gault method, and 12-lead ECG within reference ranges at Screening and Day-1 (See Sections 8.1 and 8.2 and Appendix B, Table 2, Table 3, and Table 4).
   - **Note 2**: Exceptions to BP, HR and laboratory test values being with normal ranges are:
     - Subjects with baseline HR ≥ 45 to 50 bpm may be accepted if otherwise healthy adults with known history of asymptomatic bradycardia.
     - Subjects with baseline SBP up to 140 mmHg and DBP up to 90 mmHg may be accepted if otherwise healthy.
     - A laboratory value that is Grade 1 will be allowed if not considered to be clinically significant by the investigator, with the exception of ALT, AST, AP, total and direct bilirubin, BUN, serum creatinine, CL_{CR}, and urine protein.
6. Sexually active females must be of non-childbearing potential or must use a highly effective method of birth control from screening to 30 days following the last dose of study product.
   - **Note 1**: A female is considered of childbearing potential unless post-menopausal (defined as history of ≥1 year of spontaneous amenorrhea and a FSH level >40 IU/L), or permanently surgically sterilized.
   - **Note 2**: Highly effective contraceptive methods include: (a) surgical sterilization methods, such as tubal ligation, bilateral oophorectomy, salpingectomy, hysterectomy, or successful tubal obliteration (e.g., Essure®) with documented

radiological confirmation test at least 90 days after the procedure, or (b) long-acting reversible contraception, such as progestin-releasing subdermal implants, copper intrauterine devices (IUDs), levonorgestrel-releasing IUDs.

- **Note 3**: A subject who is not sexually active and abstains from sexual intercourse can be enrolled and abstinence documented.

7. Sexually active males must be vasectomized or agree to use barrier contraception and not donate sperm from first dose of study product until 30 days following the last dose.
   - **Note 1**: Barrier contraception includes use of condom with spermicide.
   - **Note 2**: A subject who is not sexually active and abstains from sexual intercourse can be enrolled and abstinence documented.
8. Subjects must be willing to avoid excessive physical exercise within 48 h prior to dosing until discharge from the clinical trial unit (CTU) on Day 8, and 24 h before the last visit (Day 11 + 3 days).
9. No history of acute febrile or infectious illness for at least 7 days prior to the administration of study drug(s).

5.2 Subject Exclusion Criteria

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

1. Known history of a clinically significant food or drug allergy/hypersensitivity including known allergy/hypersensitivity to ERT, any β-lactam drugs or other related drugs.
2. Current seasonal allergies with ongoing symptoms for more than a week prior to dosing requiring glucocorticoids and/or frequent use of antihistamines for treatment.
3. Any history of a chronic condition including renal failure that may increase risk to subject or interfere with endpoint assessment, or any unstable chronic disease.
   - **Note 1**: Unstable chronic disease is defined by need for frequent medical interventions that lead to a change in medications and/or required hospitalization, surgery or an invasive procedure or emergency department/urgent care visit, as determined by the Site PI.
   - **Note 2**: Any chronic disease, that has been diagnosed within 90 days of screening is excluded.
4. History of any psychiatric condition that has required hospitalization in the last 12 months or subject is considered psychologically unstable by the investigator.
5. History of any clinically significant (CS) disease or disorder, medical/surgical procedure, or trauma within 4 weeks prior to initiation of administration of study product(s).
6. History of *Clostridium difficile* induced diarrhea within 1 year before screening
7. Known history of past or current epilepsy or seizure disorders, excluding febrile seizures of childhood.
8. Prior exposure to ZID.

9. Use of any prohibited prescription or non-prescription medication within 14 days prior to the first dose of study drug(s) as described in Section 6.6.
10. Use of any investigational drug product within 30 days or 5 half-lives (whichever is longer) before investigational product administration in this study.
11. Planned participation in a clinical research study that requires treatment with a study drug, blood draws or other invasive assessments during the study period (screening until final visit).
12. Blood or plasma donation of 500 mL within 3 months or more than 100 mL within 30 days before signing informed consent or planned donation prior to completion of this trial.
13. Positive serum pregnancy test for women at screening and urine pregnancy test at check-in.
14. Positive urine alcohol test or urine drug screen test at screening or check-in (Day -1).
15. Positive test for HIV antibodies, hepatitis B-virus surface antigen (HBsAg), or anti-hepatitis C-virus antibodies (anti-HCV) at screening.
16. History of ≥10 pack-years smoking in the 5-year period before screening, or positive urine cotinine screen at check-in.
    - **Note 1:** Nicotine products include cigarettes, e-cigarettes, pipe, cigar, chewing tobacco, nicotine patch.
    - **Note 2:** Positive urine cotinine at screening is allowed if negative at check-in (Day -1).
17. History of binge drinking or heavy drinking of alcohol at any time in the 6 months before study product administration.
    - **Note 1**: Binge drinking is defined as 5 or more drinks during single occasion if male, or 4 or more if female.
    - **Note 2**: Heavy drinking of alcohol is defined as consumption of more than 15 units of alcohol per week if male, or more than 8 units if female.

5.3 Treatment Assignment Procedures

5.3.1 Enrollment and Randomization Procedures

Fifty-two healthy subjects who consent to participate in the trial and meet the eligibility criteria will be enrolled following admittance to the CTU and confirmation of eligibility. Subjects will be registered using Advantage eClinical®, a web-based application developed by The Emmes Company, LLC, the DMID Statistical and Data Coordinating Center (SDCC) for the trial. Reasons for screen failure will be entered in Advantage eClinical®. Instructions for using the enrollment module are included in the Advantage eClinical® User's Guide.

For the drug combination cohorts (Cohorts 1, 4 and 7) and standalone ERT cohorts (Cohorts 2 and 5), the trial will be double-blinded, placebo controlled, with eight subjects randomized to receive either active drug(s) or placebo (normal saline) in an overall 3:1 ratio (6 subjects study drug and 2 subjects placebo). For each drug combination dosing cohort, two sentinel subjects

will be randomized in a 1:1 fashion to active drug and placebo and will be followed for 24 h. If there are no safety events, the remaining 6 subjects in each cohort will be randomized in a 5:1 ratio to active drug and placebo. Sentinel subjects will not be used for the randomized, standalone ERT cohorts and for the ZID cohorts.

Randomized treatment assignments for these cohorts will be generated centrally at SDCC through the Advantage eClinical® application by the unblinded study biostatistician, and a list will be transferred to the unblinded study personnel (i.e., research pharmacists and a verifier) at the research site prior to start of the study for the purpose of an emergency back-up, which will be kept up in a secure place. The research pharmacists will perform dose preparation; the unblinded verifier is a member of the pharmacy team who will not participate in dose preparation.

In the standalone ZID cohorts (Cohorts 3 and 6), 6 subjects will be enrolled, and all will receive active drug. All subjects will be registered into the Advantage eClinical®.

5.3.2 Masking Procedures

All investigative site personnel, study volunteers, DMID, Wockhardt and DVC personnel will remain blinded through database lock, with the exception of an unblinded pharmacist/verifier at the research site, an unblinded SDCC biostatistician, and a DMID/CMS manager responsible for PK sample storage.

The randomization scheme will be provided to the unblinded research pharmacist at the site, who will perform dose preparation, and a pharmacy verifier who will not participate in dose preparation. These pharmacy personnel at the site will not be involved in study-related assessments or have subject contact for data collection following study drug administration.

The study staff participating in the administration of study product and assessment of the subjects will not be aware of the administered contents of the IV infusion in the randomized arms of the study. Study drugs will be diluted in normal saline, and they will look identical to placebo in the IV infusion bag so the study staff and the subject will not be able to determine whether placebo or active drug are being administered. The label on the IV infusion bag will not have information that can identify the contents.

Only plasma and urine PK samples collected from subjects who received active drug(s) will be sent to the bioanalytical laboratory, KCAS, for testing. To select these samples, an SDCC Unblinded Biostatistics Team will determine which volunteers received active drug and will provide the list of samples from those volunteers to DMID-CMS. DMID-CMS repository employees will identify those samples and ship to KCAS. KCAS personnel are blinded. Individual samples are barcoded, and the barcode includes information that can match the

sample to the volunteer identification number, aliquot number, treatment cohort and collection timepoint. Data from KCAS will be received by the SDCC Unblinded Biostatistics Team and entered into the database after the safety analysis was completed by the SDCC Blinded Biostatistics team.

The SMC will receive blinded data in aggregate and presented by cohort. The SMC may review unblinded data prepared by an unblinded biostatistician in the closed session only.

5.3.3 Reasons for Withdrawal and Discontinuation of Study Drug Administration

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

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

- Request by the subject to terminate participation.
- Failure to receive the study drug due to difficulty initiating or maintaining an intravenous infusion line.

A subject may be removed from the trial after dosing for the following reasons; however, whenever possible, the subject will be followed for safety per protocol to Day 11 (+ 3 days):

- Failure to adhere to protocol requirements.
- Loss to follow-up.
- Request of primary care provider.
- Request of the Institutional Review Board (IRB)/Ethics Committee (EC), FDA or DMID.
- The occurrence of a Serious AE (SAE) or treatment-emergent AE warranting withdrawal.
- Any other condition that the study site PI judges to unduly increase the risk to the subject.

5.3.4 Handling of Withdrawals and Discontinuation of Study Drug Administration

- Subjects who are withdrawn before dosing or subjects who do not have at least 1 PK blood draw should be replaced with a subject assigned to the same cohort and treatment as the subject they replaced.
- Additionally, subjects will be replaced in each dosing cohort to ensure there are at least seven subjects with complete PK profiles in each 8 subject dosing cohort or 5 subjects in each 6 subject dosing cohort. A Subject will be considered to have a complete PK profile if ≥70% of plasma PK samples are collected. (See the SAP for details).
- Subjects who withdraw after receiving all study doses and completed scheduled assessments to Day 8 will not be replaced unless more than 50% of subjects in a cohort are withdrawn from the trial.

- Replacement of additional withdrawals is at the discretion of the Investigator in consultation with the Sponsor. Replacement subjects should be allocated to the same treatment as the subject they replaced.

Generally, subjects who received any amount of the study drug but withdraw from the trial will be encouraged to continue follow-up (with subjects' consent) for safety assessments until Final Visit (Day 11+3) and for completion of scheduled plasma PK sample collection following the last dose of study drug they received. Subjects withdrawing will be asked to complete safety assessments according to the ET schedule (See Section 7.6).

5.3.5 Lost to Follow-up

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

5.3.6 Termination of Study

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

6 STUDY INTERVENTION/INVESTIGATIONAL PRODUCTS

6.1 Description of Study Products

6.1.1 Zidebactam for Injection, 1 g/vial

Zidebactam dihydrate (CAS No. 1996664-59-5) is a proprietary New Chemical Entity (NCE) of Wockhardt Bio AG. It is an entity from the diazabicyclooctane (DBO) class and exhibits a novel mode of action as a β-lactamase inhibitor and β-lactam enhancer. It is chemically identified as (2S, 5R)-7-Oxo-6-sulphooxy-2-[N'-((R)-piperidin-3-carbonyl)-hydrazinocarbonyl]-1, 6-diaza-bicyclo [3.2.1] octane dihydrate. The structure and absolute stereochemistry of ZID have been confirmed though various instrumental methods of analysis. ZID is slightly hygroscopic and is freely soluble in water and insoluble in dichloromethane. ZID (WCK 5107) drug substance is manufactured at Wockhardt Limited, Ankleshwar, Gujarat, India and/or Aragen Life Sciences Private Ltd (formerly GVK Biosciences Pvt. Ltd and Inogent Laboratories Private Ltd.), Hyderabad, Telangana, India. ZID for Injection 1g/vial drug product is manufactured at Wockhardt Limited, Shendra, Aurangabad, India [34].

6.1.2 Ertapenem Sodium for Injection, 1 g/vial (ERT)

ERT is a sterile, synthetic, parenteral, 1-β methyl-carbapenem that is structurally related to beta-lactam antibiotics. It is generally available as ertapenem sodium (CAS No. 153773-82-1), and chemically identified as [4R-[3(3S*,5S*),4α,5β,6β(R*)]]-3-[[5-[[(3-carboxyphenyl)amino] carbonyl]-3-pyrrolidinyl]thio]-6-(1-hydroxyethyl)-4-methyl-7-oxo-1-azabicyclo[3.2.0] hept-2-ene-2-carboxylic acid monosodium salt. ERT sodium is a white to off-white hygroscopic, weakly crystalline powder. It is soluble in water and 0.9% sodium chloride solution, practically insoluble in ethanol, and insoluble in isopropyl acetate and tetrahydrofuran [34]. For this study, a U.S. FDA- approved generic ERT for injection will be procured from Dr. Reddy's Laboratories, Inc., in the U.S. by Wockhardt (37).

6.1.3 WCK 6777 (ERT-ZID)

It is a combination of ERT and ZID (WCK 5107). For this clinical trial, single use, sterile, lyophilized *ZID for Injection, 1 g/ vial* and *ERT Sodium for Injection, 1 g ertapenem/vial* will be used and supplied separately by Wockhardt. Solutions for injection containing ZID and ERT will be reconstituted in the Pharmacy of the clinical research site in the desired drug doses.

6.1.4 Placebo

0.9% Sodium Chloride Injection, USP will be used as the placebo for this study.

Sterile Water for Injection, USP is sterile, nonpyrogenic, distilled water for intravenous administration after addition of a suitable solute. No antimicrobial or other substance has been added. The pH is 5.5 (5.0 to 7.0). The osmolarity is 0 mOsmol/L. It will be used for reconstituting ERT and ZID separately or combined (for WCK 6777) prior to dilution with sterile 0.9% Sodium Chloride Injection, USP.

0.9% Sodium Chloride Injection, USP is a sterile, nonpyrogenic solution. Each 100 mL contains 900 mg sodium chloride in water for injection. It contains sodium (Na^+) 154 mEq/L and chloride (Cl^-) 154 mEq/L. The osmolarity is 308 mOsmol/L (calc.). The solution may contain hydrochloric acid and/or sodium hydroxide for pH adjustment (pH 5.6, range 4.5 to 7.0). This solution contains no bacteriostatic, antimicrobial agent or added buffer. It will be used as the secondary diluent for ERT and ZID and as the placebo in this study.

6.1.5 Acquisition

ZID for Injection, 1 g/vial will be provided by Wockhardt. ERT Sodium for Injection, 1 g ertapenem/vial will be procured by Wockhardt from a US supplier [37]. Upon request by DMID, study products will be shipped for clinical labeling to the DMID Clinical Materials Services (DMID-CMS) at the address provided in the protocol-specific MOP.

The study drugs will be shipped from DMID-CMS to the CTU upon request and approval by DMID. Details will be provided in the protocol-specific MOP.

Sterile Water for Injection, USP and 0.9% Sodium Chloride Injection, USP will be purchased by the clinical research site from commercial sources. Details will be described in the protocol-specific MOP.

Ancillary supplies required for IV administration (including syringes or infusion bag, tubing and IV catheter and line-filter) will be supplied by the clinical research site as described in the protocol-specific MOP.

6.1.6 Formulation, Packaging, and Labeling

For this Phase 1 clinical study, individual vials of Zidebactam for Injection 1 g / vial and Ertapenem Sodium for Injection, 1 g ertapenem/ vial will be used.

- Zidebactam for Injection, 1 g/vial will be used in this study. The drug product is manufactured by Wockhardt Limited, Shendra, Aurangabad, India according to cGMP requirements as a sterile, lyophilized powder. Zidebactam for Injection is packed in 20 mL USP Type I clear tubular glass vial, stoppered with 20 mm rubber stopper and

sealed with aluminum flip-off seal. Product information, packaging and labeling will be described in the MOP.

- Ertapenem Sodium for Injection, 1 g ertapenem/vial is supplied in single dose glass vials as a sterile, lyophilized powder. Each vial contains 1.046 grams of ERT sodium, equivalent to 1 gram of ERT. The sodium content is approximately 137 mg (approximately 6.0 mEq). Each vial of ERT sodium contains the following inactive ingredients: 175 mg sodium bicarbonate and sodium hydroxide to adjust pH to 7.5 [37]. Commercial source, product information packaging and labeling will be described in the MOP.
- WCK 6777 for Injection (combination of ZID [WCK 5107] and ERT) will be formulated for IV infusion at the clinical research pharmacy of the CTU using separate vials of ZID for Injection, 1 g/vial and ERT Sodium for Injection, 1 g ertapenem/vial to the desired doses. (See Section 6.2)
- Sterile Water for Injection, USP - Commercial source, product information and packaging will be described in the MOP.
- 0.9% Sodium Chloride Injection, USP - Commercial source, product information and packaging of the diluent will be described in the MOP.

Each product vial and diluent will be labeled in compliance with applicable regulatory requirements as described in the MOP and will include FDA-required cautionary statement, "Caution – New drug - Limited by United States Federal Law to Investigational Use."

6.1.7 Product Storage and Stability

6.1.7.1 Stability in Storage Prior to Reconstitution

It is recommended that the vials for both drug products should be stored refrigerated at 2°C to 8°C (35.6°F to 46.4°F) within the packaging until time of preparation for dosing.

- ZID for Injection, 1 g/vial - Based on available stability data, the proposed shelf life for ZID for Injection, 1 g/vial is 48 months at 2°C to 8°C (35.6°F to 46.4°F). Further stability study is on-going up to 60 months at long-term conditions. GMP/ Clinical batch of ZID for Injection 1 g/vial (#QV10001 Mfg. date: Dec. 2019), which is intended to be used in this WCK 6777 phase 1 study, was found to be stable up to 24 months at accelerated conditions (25°C± 2°C / 60% ± 5% RH) and 36 months at long-term conditions (5°C± 3°C). Extended stability testing is ongoing and proposed "Use by Date" will be based on satisfactory stability data and ICH Q1E recommendation. Details will be provided in the study-specific MOP.

- ERT Sodium for Injection, 1 g ertapenem/vial lyophilized powder should not be stored above 25°C (77°F) before reconstitution per product information [37]. For this study, it is recommended that lyophilized ERT be stored refrigerated at 2°C to 8°C (35.6°F to 46.4°F) within the packaging until time of preparation for dosing. The expiration date of ERT for Injection 1 g/vial shall be referred from the labelling of the commercial product being procured from the US market.
- Sterile Water for Injection, USP will be stored per manufacturing instructions. Product storage and stability will be described in the MOP.
- The diluent, 0.9% Sodium Chloride Injection, USP, will be stored per manufacturing instructions. Product storage and stability will be described in the MOP.

6.1.7.2 Stability of ERT or ZID after Reconstitution in Sterile Water for Injection

The reconstitution of ERT or ZID vial should start within 1 h after removal from refrigeration.

The reconstituted ERT vials should be immediately diluted in 0.9% Sodium Chloride for the preparation of either the ERT standalone infusion solution or the WCK 6777 (ERT+ZID) infusion solution.

Likewise, the reconstituted ZID vials should be immediately diluted in 0.9% Sodium Chloride for the preparation of either the ZID standalone infusion solution or the WCK 6777 (ERT+ZID) infusion solution.

6.1.7.3 Stability of ERT or ZID after Dilution in 0.9% Sodium Chloride

- ERT or ZID or ERT-ZID infusion solutions in 100 mL (for Cohort 1 - ERT-ZID 1 + 1 g) or 250 mL (for remaining Cohorts 2 to 7) were found to be stable for up to 6 hours at room temperature (25 ± 2°C) and up to 24 hours at refrigeration (5 ± 3°C).

In spite of the above-mentioned stability of ERT or ZID or WCK 6777 in 0.9% normal saline, to negate the risk of sterility loss uniformly for all the cohorts, the infusion (stored at room temperature or refrigerated) should begin within 2 h of preparation.

6.1.7.4 Photostability

- ZID for Injection, 1 g/vial is photostable
- ERT Sodium for Injection, 1 g ertapenem/vial is photostable

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

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

The site Research Pharmacist (RP) will prepare the study product on the same day as administration as instructed in above sections. The study drug will be inspected for damage, contamination, discoloration, or particulate matter before use. Any study drug that fails inspection will be quarantined at appropriate temperature and labeled 'Do Not Use' until further notice. The Site Principal Investigator (PI) or responsible person will immediately contact the DMID Clinical Project Manager (CPM) and study clinical team for further instructions before administering any additional study drug infusions. Based on the information collected, DMID, with consultation from Wockhardt, will determine whether the affected study drug can be used. If it cannot be used, the CTU will receive specific instructions on how to return it to DMID CMS or destroy it on site.

The site Research Pharmacist will prepare the infusion using aseptic technique under a sterile environment (e.g., Biologic Safety Cabinet or laminar flow hood) as described in the protocol-specific MOP.

The appropriate number of vials will be removed from storage to prepare the infusion. Each vial of ERT Sodium, 1 g ertapenem/vial and/or ZID for Injection, 1 g/vial, depending on the treatment cohort, will be reconstituted separately using 5 mL sterile Water for Injection as the primary diluent for each ZID 1-g vial and 10 mL sterile Water for Injection as the primary diluent for each ERT Sodium, 1-g vial. A 0.9% Sodium Chloride Injection solution will serve as the secondary diluent to make up the total volume for IV infusion to 100 mL for Cohort 1 or 250 mL for Cohorts 2-7.

The IV administration set must contain a 0.20 μm in-line filter.

Refer to protocol-specific MOP for detailed information on study product dose and volume calculations, documentation of time of placement and removal from refrigeration as well as preparation and administration of the study product and handling of infusion interruptions.

6.3 Modification of the Study Intervention/ Investigational Products

6.3.1 Overdose

An overdose is defined as a dose greater than the high-dose level evaluated in the trial. Intentional overdosing of ERT or ZID is unlikely.

All overdoses will be reported as a deviation; if the overdose is associated with a TEAE, then the TEAE will also be reported. In the event of an overdose, the Investigator will use clinical judgment in treating the overdose and contact the DMID Medical Monitor (MM). There is no specific information available on the treatment of overdosage with ERT or ZID.

Intravenous administration of ERT at a dose of 2 g over 30 min or 3 g over 1h to 2h in healthy adult volunteers resulted in an increased incidence of nausea [29]. In clinical trials in adults, inadvertent administration of three 1 g doses of ERT in a 24-hour period resulted in diarrhea and transient dizziness in one patient [29]. In the event of an overdose, ERT should be discontinued, and general supportive treatment given until renal elimination takes place. In case of severe overdosage, especially in patients with compromised renal function, hemodialysis will aid in the removal of ERT from the body [28].

There is no information on overdose with ZID.

The investigator will refer to the relevant document(s) for detailed information regarding warnings, precautions, contraindications, TEAEs, and other significant data pertaining to the study drug. Such documentation may include but not be limited to the IB.

6.4 Accountability Procedures for the Study Intervention/ Investigational Products

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

All study drugs, whether administered or not, will be documented on the appropriate study drug accountability record or dispensing log. Used and unused study drug vials will be retained until monitored. Upon completion of the trial and after the final monitoring visit, any remaining unused study drugs will either be returned or destroyed appropriately at the CTU as per DMID requirements and instructions that will be communicated to the CTU by the DMID CPM. Unused study drug vials will be released for disposition per DMID requirements. DMID does not require used containers of study product to be maintained at the research pharmacy, except when the local institution's SOP/policy mandates retaining used IV vials. If local SOPs allow destruction of used study product containers, the used vials can be destroyed per the site's SOPs; a second staff member must observe the destruction and sign verification (two signatures) that the used vials were discarded.

Any unused solution left in the IV infusion syringe or bag or the IV administration tubing after administration to the subject should be discarded as biohazardous waste. If a container of study product is unusable due to breakage, or any other reason, this explanation should also be noted on the Study Product Accountability Record (e.g., broken – dropped on floor). Details will be provided in the MOP.

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

Since each dose of study drug(s) will be administered by site personnel, subject compliance is not anticipated to be an issue. Complete information regarding any partial or interrupted dosing will be documented. Subjects unable to receive the full volume of study drug(s) infusion will be withdrawn from data analysis and followed up for safety as described in Section 5.3.3.

6.6 Prior and Concomitant Medications/Treatments

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

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

Prior prescription medications will be recorded at Screening Visit. All prior medications are not allowed during the study period with the exception of oral contraceptives, which are permitted throughout the study if already used.

The following medications are prohibited for the indicated periods prior to dosing:

- Receipt of probenecid or furosemide within 14 days prior to study enrollment.
- Receipt of any antibiotics within 14 days prior to study enrollment.
- Receipt of prescription medications (except birth control pills or hormone replacement in females) within 14 days prior to study enrollment, unless in the opinion of site investigator the medication will not interfere with the study procedures or impact subject safety).
- Receipt of the following medications that interact with human OAT3 within 14 days prior to study enrollment.

  Note: Adefovir, Anagliptin, Baricitinib, Cefaclor, Cimetidine, Ciprofloxacin (Systemic), Clofarabine, Eluxadoline, Empagliflozin, Furosemide, Ketoprofen, Methotrexate, Mycophenolate, PEMEtrexed, Penicillin G (Parenteral/Aqueous), Penicillin G Benzathine, Penicillin G Procaine, Penicillin V Benzathine, Penicillin V Potassium, Zidovudine.
- Receipt of herbal and dietary supplements (including St. John's Wort) within 14 days prior to study enrollment.
- Receipt of valproic acid within 14 days prior to study enrollment.

Non-prescription medications, herbs, vitamins, and nutritional supplements will not be taken within 14 days before dosing and during the trial. Exceptions: vitamins and OTC medications, taken for <48 h for the treatment of common symptoms (e.g., headache, indigestion, muscle pain), including solitary doses of up to 1,000 mg acetaminophen (paracetamol), may be allowed if approved by the Site PI or authorized clinician(s).

Blood/blood products (RBCs, WBCs, platelets, and plasma) donation of 500 mL within 3 months or more than 100 mL within 30 days before signing informed consent to this trial is not allowed, and it is prohibited during the course of this trial.

Following dosing, each new ConMed and changes to existing medications will be recorded. Subjects will be required not to utilize non-study medications during the trial except those deemed necessary by the Site PI or authorized clinician(s).

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

6.7 Subject Restrictions and Precautions

From signing informed consent until end-of-study, the subjects have to follow the instructions of the study site. They have to adhere to the following restrictions during participation in the study.

6.7.1 Smoking

Smoking is prohibited during the in-patient period of the study (Day -1 to discharge on Day 8). Subjects will be counseled to avoid smoking during the out-patient follow-up period until the last visit (Day 11 + 3 days).

6.7.2 Physical Activity

Subjects will be counseled to refrain from excessive physical exercise starting 2 days before dosing until discharge from the CTU on Day 8, and 24 hours before the last visit (Day 11 + 3 days).

6.7.3 Food and Fluid intake

Subjects will be provided food and non-caffeinated and non-alcoholic beverages by the CTU during the dosing period of the trial. Subjects will fast at least 4 hours prior to blood draw on days of clinical laboratory testing (screening, Day -1, Day 2, Day 4, Day 6, Day 8 and Day 11

+ 3 days (last visit)). Subjects will fast for at least 4 h before dosing on Day 1 and 1 h after end of infusion. Water can be taken ad lib during those periods of fasting. Food may be eaten 1 h after the end of infusion.

6.7.4 Alcohol, Marijuana and Illicit Drugs

Alcohol will not be served during the in-patient period from Day -1 check-in to Day 8. Marijuana and illicit drugs are prohibited during the course of the trial.

7 STUDY SCHEDULE

The Schedule of Study Procedures and Evaluations is included as Appendix A. Clinical and Laboratory Procedures are described in Section 8.

7.1 Recruitment

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

7.2 Screening Visit (Day -28 to Day -2)

This will be an out-patient visit to complete the following within 28 days before study drug dosing:

Note: A second screening visit may be scheduled to complete screening tests to accommodate subjects' personal schedule.

- Obtain informed consent.
- Assign a study ID number to subjects who consent to participate.
- Record demographics including age, gender, race, and ethnicity.
- Obtain contact information.
- Obtain height (ht) and body weight (wt) and calculate Body Mass Index (BMI; wt [kg] / ht [m^2]).
- Take VS.
- Obtain Medical History (MH).
- Review history of Prior Medications.
- Perform complete Physical Examination (PE).
- Obtain fasting (at least 4 hours) blood and urine samples for screening clinical laboratory tests that include a calculation of creatinine clearance (CL_{CR}) by the Cockcroft-Gault equation.
- Obtain blood samples for viral serology (HIV antibody, HBsAg, HCV antibody).
- Obtain serum for β-HCG pregnancy test from all women.
- Obtain serum for FSH level only from post-menopausal women.
- Obtain urine sample for illicit drugs and drugs of abuse (urine drug screen), cotinine and alcohol.
- Obtain a triplicate 12-lead ECG with 10-sec rhythm strip.
- Counsel on use of contraception (males and females), avoidance of pregnancy (women of childbearing potential), avoidance of excessive physical exercise, avoidance of prohibited medication, illicit drugs, alcohol, and nicotine products.

- Confirm eligibility.

Subjects who meet the eligibility criteria will be contacted by CTU personnel and asked to return on Day -1 to complete check-in assessments and be admitted to the CTU if they continue to meet eligibility criteria. Subjects will be reminded to avoid excessive physical exercise, prohibited medication, illicit drugs, alcohol and nicotine products, and too fast for at least 4 h before the Day -1 visit,

Subjects who fail screening due to a medical condition or abnormal laboratory tests including pregnancy test and positive tests for HIV, HBV and HCV will be informed of the findings and counseled to seek medical care for further evaluation and treatment. If subjects test positive for HIV antibody, HBsAg, and/or HCV antibody, they will be informed that test results may be reported to the local health authorities according to state or local law.

Subjects who fail screening assessments will not be rescreened unless they had an inter-current, short-term medical illness per site PI or authorized study clinician (listed on FDA Form 1572) discretion. Subjects who meet eligibility criteria but could not be enrolled within permissible window may be rescreened.

7.3 Baseline / Check-in (Day -1)

Subjects meeting all inclusion and no exclusion criteria at Screening Visit will check into the CTU on Day -1 and the following procedures will be performed:

- Review inclusion/exclusion criteria to confirm the subject remains eligible for enrollment.
- Update MH.
- Update Prior Medications.
- Obtain VS.
    - **Note:** Obtain VS before blood draws
- Obtain body weight.
- Perform complete PE.
- Obtain blood and urine samples for clinical laboratory tests that include a calculation of CL_{CR} by the Cockcroft-Gault equation.
- For all women, a urine β-HCG pregnancy test will be done, and negative results confirmed before dosing.
- Obtain urine sample for illicit drugs and drugs of abuse (urine drug screen), cotinine, and alcohol.
- Obtain baseline 12-lead standard ECG with 10-sec rhythm strip.
- Admit eligible subjects in the CTU.

- Assign to study treatment cohort and randomize to active drug or placebo, except for Cohorts 3 and 6.
- Counsel to avoid excessive physical exercise, encourage good hydration.

7.4 Inpatient Treatment Period (Days 1 to 8)

The following assessments will be performed daily before and after dosing on study Days 1 to 7, and before discharge from the CTU on Day 8.

7.4.1 DAY 1

2+ hours Before Dosing:

- Withhold solid food at least 4 h before dosing.
- Allow access to water and non-carbonated beverages.
- Review inclusion/exclusion criteria to confirm the subject remains eligible for dosing.
- Update MH.
- Review any new medications and update list of prior medications as needed.
- Perform symptom-directed (focused) PE as needed for evaluation of new symptoms.

About 1 – 2 hours Before Dosing:

- Insert IV line for study drug infusion into a forearm vein
- Insert IV catheter for blood collection into a vein in the other arm

About 30 – 60 min Before Dosing:

- Obtain VS within 30 min before dosing (baseline).
- Obtain pre-dose blood (plasma) PK sample for total and free study drug within 30 min before starting administration of study drug.
- Obtain pre-dose urine PK sample for study drug concentration within 30- 60 min before starting drug administration.
    - Void bladder as close to study drug administration as possible.

Dosing:

- Administer study drug as a single IV infusion with an infusion pump in a forearm vein. The volume and duration of infusion will vary according to study cohort as follows:
    - For Cohort 1: 100 mL, 30 (±5) minutes;
    - For Cohort 2, Cohort 3 and Cohort 4: 250 mL, 60 (±10) minutes; and
    - For Cohort 5, Cohort 6 and Cohort 7: 250 mL, 120 (±10) minutes**.**

After Dosing Initiation:

- Initiate AE assessments until the end of the study.
- Perform symptom-directed (focused) PE as needed (for TEAE assessments).

- Initiate recording of Concomitant Medications (ConMeds).
- Withhold solid food until at least 1 h after end of infusion.
- Provide access to water and non-carbonated beverage.
- Check infusion site for infusion site reactions during the infusion, at the end-of-infusion (+10 min), 1 h (±10 min) after the end of infusion, and as needed for evaluation of local symptoms at the infusion site. Replace as needed. (Details will be provided in the MOP.)
- Check IV blood draw lines and replace as needed.
- Obtain VS at the end of dosing (+10 min), and 1 h (±5 min) after the end of dosing.
  - **Note 1**: *More frequent monitoring will be at the PI's or authorized study clinician's discretion based on subject's clinical status.*
  - **Note 2:** Obtain VS before blood draws.
- Obtain blood (plasma) PK samples for total and free study drug as follows:
  - From Cohort 1 (infusion duration 0.5 h): within 30 min before dosing, and at 0.25 h (±5 min), 0.5 h (±5 min) [immediately at end of infusion], 1 h (±5 min), 2 h (±5 min), 3 h (±10 min), 4 h (±10 min), 8 h (±15 min), 12 h (±15 min), and 18 h (±15 min) after the start of the infusion.
  - From Cohorts 2, 3, and 4 (infusion duration 1 h): within 30 min before dosing, and at 0.5 h (±5 min), 1 h (±5 min) [immediately at end of infusion], 2 h (±5 min), 3 h (±10 min), 4 h (±10 min), 8 h (±15 min), 12 h (±15 min), and 18 h (±15 min) after the start of the infusion.
  - From Cohorts 5, 6 and 7 (infusion duration 2 h): within 30 min before dosing and at 1 h (±5 min), 2 h (±5 min) [immediately at end of infusion], 3 h (±10 min), 4 h (±10 min), 8 h (±15 min), 12 h (±15 min), and 18 h (±15 min) after the start of the infusion.
- Obtain post-dose urine PK samples at the following intervals after the start of the infusion: 0 to 4 h (±10 min), 4 to 8 h (±15 min), 8 to 12 h (±15 min), and 12 to 24 h (±30 min).
- Counsel to avoid excessive physical exercise, encourage good hydration.

7.4.2 DAY 2

As for DAY 1 with the ***exception*** that frequent plasma and urine PK samples are not collected after dosing.

<u>Before Dosing</u>:

- Check the infusion site for any reactions.
- Assess the IV lines used for infusion and blood PK draws and replace as needed.
- Obtain VS within 30 min before dosing.

- Obtain blood for safety laboratory testing (Hematology [HEM], Coagulation [COAG], and Clinical chemistry [CHEM] that includes a calculation of CL_{CR} by the Cockcroft-Gault equation 30-60 min before dosing.
- Collect blood (plasma) PK sample at 24 h (±30 min) after initiation of dosing on Day 1.
  - **Note***: If collected within 30 min prior to dosing, this sample could also be the "pre-dose" sample for Day 2.*
- Complete collection of the 12 h to 24 h (±30 min) urine PK sample after dosing on Day 1 before dosing on Day 2.

Dosing:

- Administer study drug at approximately the same time (within 24 h +1h) as on Day 1.
- The duration of infusion will vary according to study cohort as described on Day 1.

After Dosing Initiation:

- Check infusion site for infusion site reactions as on Day 1.
- Assess the IV lines used for infusion and blood PK draws and replace as needed.
- Obtain VS at the end of dosing (+10 min), and 1 h (±5 min) after the end of dosing.
  - **Note 1**: *More frequent monitoring will be at the PI's or authorized study clinician's (listed on FDA Form 1572) discretion based on subject's clinical status.*
  - **Note 2:** Obtain VS before blood draws
- Perform AE assessments.
- Perform symptom-directed (focused) PE as needed for TEAE assessments.
- Document ConMeds.
- Provide solid food at least 1 h after the end infusion.
- Collect blood (plasma) for PK 12 h (± 15 min) after the start of infusion.
- Counsel to avoid excessive physical exercise, encourage good hydration.

7.4.3 DAY 3

As for DAY 1 with the ***exception*** that frequent plasma and urine PK samples are not collected after dosing.

Before Dosing:

- Check the infusion site for any reactions.
- Assess the IV lines used for infusion and blood PK draws and replace as needed.
- Obtain VS within 30 min before dosing.
  - **Note:** Obtain VS before blood draws.
- Collect pre-dose blood (plasma) PK sample within 30 min before starting administration of study drug.

Dosing:

- Administer study drug at approximately the same time (within 24 h +1h) as on Day 1.
- The duration of infusion will vary according to study cohort as described on Day 1.

After Dosing Initiation:

- Check infusion site(s) for infusion site reactions as on Day 1.
- Obtain VS at the end of dosing (+10 min), and 1 h (±5 min) after the end of dosing.
  - **Note 1**: *More frequent monitoring will be at the PI's or authorized study clinician's discretion based on subject's clinical status.*
  - **Note 2:** Obtain VS before blood draws.
- Perform AE assessments.
- Perform symptom-directed (focused) PE as needed for TEAE evaluation of TEAEs.
- Document ConMeds.
- Provide solid food at least 1 h after the end infusion.
- Collect blood (plasma) for PK 12 h (± 15 min) after the start of infusion.
- Counsel to avoid excessive physical exercise, encourage good hydration.

7.4.4 DAY 4

As for DAY 1 with the ***exception*** that frequent plasma and urine PK samples are not collected after dosing.

Before Dosing:

- Check the infusion site for any reactions.
- Assess the IV lines used for infusion and blood PK draws. Replace IV catheters as needed.
- Obtain VS within 30 min before dosing
  - **Note:** Obtain VS before blood draws
- Obtain blood for safety laboratory testing (Hematology [HEM], Coagulation [COAG], and Clinical chemistry [CHEM] that includes a calculation of CL_{CR} by the Cockcroft-Gault equation 30-60 min before dosing.
- Obtain urine for urinalysis.
- Collect pre-dose blood (plasma) PK sample within 30 min before starting administration of study drug.

Dosing:

- Administer study drug at approximately the same time (within 24 h +1h) as on Day 1.
- The duration of infusion will vary according to study cohort as described on Day 1.

After Dosing Initiation:

- Check infusion site for infusion site reactions as on Day 1.

- Remove IV infusion catheter if it has been in use for 3 to 4 days
- Obtain VS at the end of dosing (+10 min), and 1 h (±5 min) after the end of dosing.
  - **Note 1**: *More frequent monitoring will be at the PI's or authorized study clinician's (listed on FDA Form 1572) discretion based on subject's clinical status.*
  - **Note 2:** Obtain VS before blood draws.
- Perform AE assessments.
- Perform symptom-directed (focused) PE as needed for TEAE assessments.
- Document ConMeds.
- Provide solid food at least 1 h after the end infusion.
- Collect blood (plasma) for PK 12 h (± 15 min) after the start of infusion.
- Counsel to avoid excessive physical exercise, encourage good hydration.

7.4.5 DAY 5

As for DAY 1 with the ***exception*** that frequent plasma and urine PK samples are not collected after dosing.

Before Dosing:

- Check the infusion site for any reactions.
- Assess the IV lines used for infusion and blood PK draws. Replace IV catheters as needed.
- Obtain VS within 30 min before dosing.
  - **Note:** Obtain VS before blood draws
- Collect pre-dose blood (plasma) PK sample within 30 min before starting administration of study drug.

Dosing:

- Administer study drug at approximately the same time (within 24 h +1h) as on Day 1.
- The duration of infusion will vary according to study cohort as described on Day 1.

After Dosing Initiation:

- Check infusion site(s) infusion site reactions as on Day 1.
- Obtain VS at the end of dosing (+10 min), and 1 h (±5 min) after the end of dosing.
  - **Note 1**: *More frequent monitoring will be at the PI's or authorized study clinician's (listed on FDA Form 1572) discretion based on subject's clinical status.*
  - **Note 2:** Obtain VS before blood draws.
- Perform AE assessments.
- Perform symptom-directed (focused) PE as needed for TEAE assessments.
- Document ConMeds.
- Provide solid food at least 1 h after the end infusion.

- Collect blood (plasma) for PK 12 h (± 15 min) after the start of infusion.
- Counsel to avoid excessive physical exercise, encourage good hydration.

7.4.6 DAY 6

As for DAY 1 with the ***exception*** that frequent plasma and urine PK samples are not collected after dosing.

Before Dosing:

- Check the infusion site for any reactions.
- Assess the IV lines used for infusion and blood PK draws. Replace IV catheters as needed.
- Obtain VS within 30 min before dosing.
  - **Note:** Obtain VS before blood draws.
- Obtain blood for safety laboratory testing (Hematology [HEM], Coagulation [COAG], and Clinical chemistry [CHEM] that includes a calculation of CL_{CR} by the Cockcroft-Gault equation 30-60 min before dosing.
- Collect pre-dose blood plasma PK sample within 30 min before starting administration of study drug.

Dosing:

- Administer study drug at approximately the same time (within 24 h +1h) as on Day 1.
- The duration of infusion will vary according to study cohort as described on Day 1.

After Dosing Initiation:

- Check infusion site for infusion site reactions as on Day 1.
- Obtain VS at the end of dosing (+10 min), and 1 h (±5 min) after the end of dosing.
  - **Note 1:** *More frequent monitoring will be at the PI's or authorized study clinician's (listed on FDA Form 1572) discretion based on subject's clinical status.*
  - **Note 2:** Obtain VS before blood draws
- Perform AE assessments.
- Perform symptom-directed (focused) PE as needed for evaluation of TEAEs.
- Document ConMeds.
- Provide solid food at least 1 h after the end infusion.
- Collect blood (plasma) for PK 12 h (± 15 min) after the start of infusion.
- Counsel to avoid excessive physical exercise, encourage good hydration.

7.4.7 DAY 7

All procedures and assessments are as on DAY 1.

Before Dosing:

- Check the infusion site for any reactions.
- Assess the IV lines used for infusion and blood PK draws and replace as needed.
- Obtain VS within 30 min before dosing.
- **Note:** Obtain VS before blood draws Collect pre-dose blood (plasma) PK sample within 30 min before starting administration of study drug.
- Obtain pre-dose urine PK sample within 30-60 min before starting administration of study drug.
    - Void bladder as close to study drug administration as possible.

Dosing:

- Administer study drug at approximately the same time (within 24 h +1h) as on Day 1.
- The volume and duration of infusion will vary according to study cohort as described on Day 1.

After Dosing Initiation:

- Obtain VS at the end of dosing (+10 min), and 1 h (±5 min) after the end of dosing.
    - **Note 1**: More frequent monitoring will be at the PI's or authorized study clinician's (listed on FDA Form 1572) discretion based on subject's clinical status.
    - **Note 2:** Obtain VS before blood draws.
- Obtain blood (plasma) PK samples for total and free study drug as follows:
    - From Cohort 1 (infusion duration 0.5 h): within 30 min before dosing, and at 0.25 h (±5 min), 0.5 h (±5 min) [immediately at end of infusion], 1 h (±5 min), 2 h (±5 min), 3 h (±10 min), 4 h (±10 min), 8 h (±15 min), 12 h (±15 min), 18 h (±15 min) and 24 h (± 30 min) after the start of the infusion.
    - From Cohorts 2, 3, and 4 (infusion duration 1 h): within 30 min before dosing, and at 0.5 h (±5 min), 1 h (±5 min) [immediately at end of infusion], 2 h (±5 min), 3 h (±10 min), 4 h (±10 min), 8 h (±15 min), 12 h (±15 min), 18 h (±15 min) and 24 h (± 30 min) after the start of the infusion.
    - oFrom Cohorts 5, 6 and 7 (infusion duration 2 h): within 30 min before dosing and at 1 h (±5 min), 2 h (±5 min) [immediately at end of infusion], 3 h (±10 min), 4 h (±10 min), 8 h (±15 min), 12 h (±15 min), 18 h (±15 min) and 24 h (± 30 min) after the start of the infusion.
    - Obtain post-dose urine PK samples for study drug concentration and at the following intervals after the start of the infusion: 0 to 4h (±10 min), 4h to 8h (±15 min), 8h to 12h (±15 min), and 12h to 24h (±30 min).
- Check infusion site for infusion site reactions during the infusion, at the end-of-infusion (+10 min), 1 h (±10 min) after the end of infusion, and as needed for evaluation of local symptoms at the infusion site.

- Remove IV infusion line.
- Perform AE assessments.
- Perform symptom-directed (focused) PE as needed for TEAE assessments.
- Document ConMeds
- Provide solid food at least 1 h after the end infusion.
- Counsel to avoid excessive physical exercise, encourage good hydration.

7.4.8 DAY 8

- Check the infusion and blood PK draw sites for any reactions.
- Obtain 12-lead standard ECG with 10-sec rhythm.
- Obtain VS.
- Obtain blood for safety laboratory testing (Clinical chemistry [CHEM], Hematology [HEM] and Coagulation [COAG]) that include a calculation of CL_{CR} by the Cockcroft-Gault equation) at 24 h (± 1 h) after last dose.
- Obtain urine for urinalysis.
- Collect blood (plasma) PK samples for total and free study drug at 24 h (±15 min) after initiation of study drug infusion on Day 7.
- Remove catheter for blood draws.
- Complete collection of the 12h to 24 h (±30 min) urine PK sample after dosing on Day 7.
- Perform complete PE.
- Obtain body weight.
- Perform AE assessments.
- Document ConMeds.
- Counsel on use of contraception (males and females), avoidance of pregnancy (women of childbearing potential), avoidance of excessive physical exercise 24 h before last visit (Day 11 +3 days), avoidance of prohibited medications, illicit drugs, alcohol, and nicotine products.
- Instruct on the next scheduled visit.
- Discharge subject from the CTU after review of clinical laboratory tests, ECGs and other assessments by PI or authorized study clinician.
    - **Note:** If the time of discharge is late in the evening, due to the time schedule for starting procedures on Day 7 or personal reasons (such as distance from residence), the subject may stay in the CTU overnight and be discharged the following morning.

7.5 Outpatient Follow-up Period / Last Visit (Day 11 + 3 days)

- Obtain 12-lead standard ECG with 10-sec rhythm.
- Obtain VS.
- Obtain blood for safety laboratory testing (Hematology [HEM], Coagulation [COAG], and Clinical chemistry [CHEM] that includes a calculation of CL_{CR}.
- Obtain urine for urinalysis.

- Perform complete PE.
- Check the venous infusion and blood draw sites for any reactions.
- Perform AE assessments.
- Document ConMeds.
- Counsel on use of contraception (males and females), avoidance of pregnancy (women of childbearing potential and males), and sperm donation (males) for 30 days after last dose.
- Discharge subject from the study.

7.6 Early Termination (if needed)

- Obtain 12-lead standard ECG with 10-sec rhythm.
- Obtain VS.
- Obtain blood for safety laboratory testing (Clinical chemistry [CHEM], Hematology [HEM] and Coagulation [COAG]) that include a calculation of CL_{CR}.
- Collect blood (plasma) PK sample.
- Remove any IV line present.
- Obtain urine for urinalysis.
- Perform complete PE.
- Check the infusion and blood draw site(s) for any reactions.
- Perform AE assessments.
- Document ConMeds.
- Counsel on use of contraception (males and females), avoidance of pregnancy (women of childbearing potential and males), and sperm donation (males) for 30 days after last dose.
- Discharge subject from the study.

7.7 Unscheduled Visit (if needed)

A subject may return to the clinic for an unscheduled visit at any time after discharge on Day 8. The following activities may be performed as needed:

- Obtain 12-lead standard ECG with 10-sec rhythm.
- Obtain VS.
- Obtain blood for safety laboratory testing (Clinical chemistry [CHEM], Hematology [HEM] and Coagulation [COAG]) that include a calculation of CL_{CR}.
- Collect blood (plasma) PK sample.
- Obtain urine for urinalysis.
- Check on the infusion and blood draw site(s) for any reactions.
- Perform AE assessments.
- Perform symptom-directed (focused) AE as needed for TEAE assessments.
- Document ConMeds.

8 CLINICAL PROCEDURES / EVALUATIONS

8.1 Clinical Procedures and Evaluations

8.1.1 Informed Consent

The informed consent form (ICF) will be approved by the reviewing IRB/EC and executed before performing any study-related activities.

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

8.1.2 Demographics

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

8.1.3 Inclusion/Exclusion Criteria

Eligibility screening of healthy subjects will be completed within 28 days before study drug dosing and will be documented on the subject's source documents and eCRF. Confirmation of eligibility will be performed before dosing on Day 1.

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

8.1.4 Medical History

For subjects enrolled in the trial, the medical history (MH) will be obtained by direct interview of the subject and recorded on the subject's source document and eCRF. The MH will capture the subject's current disease processes, past disease processes, history of hospitalization, history of surgery, allergies, and prior medications (see Section 6.6). Subjects will be queried regarding a history of significant medical disorders of the head, eyes, ears, nose, throat (HEENT), mouth, skin, the cardiovascular, respiratory, gastrointestinal, renal, urological, nervous, hematological, lymphatic, endocrine, musculoskeletal and genital/reproductive systems. A history of allergies including allergies to medications, medication history, and history of substance and alcohol abuse will be obtained. The MH will be obtained at Screening Visit and updated on Day -1 (Check-in) and before dosing on Day 1. After start of study drug dosing, any worsening of pre-dosing MH or new symptoms will be evaluated and reported as TEAEs.

8.1.5 Physical Examination

A complete PE – except genital, breast, and rectal exams – will be performed at the Screening Visit, check-in visit (Day -1), Day 8, and Final Visit (Day 11 + 3 days), or ET, and will assess general appearance, HEENT, heart, lungs, abdomen, skin, musculoskeletal system, lymph nodes, and a standard neurological exam.

A symptom-directed (focused) PE may be performed before dosing on Day 1, at any time after dosing on Days 1 to 7, and at Unscheduled visit for evaluation of TEAEs.

Height and weight will be measured, and BMI calculated at the Screening Visit, and weight on Day -1 and Day 8. A subject may be enrolled if BMI is ≥ 18 to 32 kg/m^2 or weight is ≥ 100 lbs.

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

8.1.6 Vital Signs

VS should be taken while resting (measured after supine for at least 5 min) and include systolic blood pressure (SBP) and diastolic BP (DBP), heart rate (HR), respiratory rate (RR) and oral temperature (T). VSs will be measured before any blood draws. BP and pulse measurement will be assessed at rest defined above with a completely automated device. Normal references ranges are shown in Appendix B, Table 2. Please reference the study specific MOP for additional detail for VS measurements.

8.1.6.1 Screening to Predose on Day 1

Abnormal VSs that are either due to technical or procedural error or the result of an acute, short-term condition as assessed by the PI (e.g., stress, anxiety, white coat syndrome) may be repeated up to twice more at rest and within at least 5 minutes of each other.

- If the second measurement is abnormal, it will be reported at the highest grade/value of severity of the two measurements and will be used for assessment of eligibility (per Inclusion Criterion #5, Note 2).
- If the second measurement is normal, a third measurement will be taken within at least 5 minutes.
- If the third measurement is normal, the subject is eligible. If the third measurement is abnormal, the value with the highest grade of severity between the first and the third measurements will be reported and will be used for assessment of eligibility (per Inclusion Criterion #5, Note 2).

8.1.6.2 Post Dosing on Day 1 through Day 11 (+ 3days) or ET

Any time after the start of the dose infusion, any abnormalities that occur either due to technical or procedural error or the result of an acute, short-term condition as assessed by the PI (e.g., stress, anxiety, white coat syndrome) may be repeated twice more at rest and within at least 5 minutes of each other.

- If the second VS is abnormal, then the highest severity grade between the first and second measurements will be used for assessment of TEAE per Appendix B, Table 2.
- If the second VS is normal, then a third repeat is to be taken at least after 5 minutes of rest.
- If the third value is abnormal, the highest grade of severity between the first and third measurements will be used for assessment of TEAE per Appendix B, Table 2.

8.1.7 12-lead Standard Electrocardiogram (ECG)

Triplicate 12-lead standard ECG and 10-sec rhythm strip will be obtained at the Screening Visit, 2 minutes apart. Single ECGs will be recorded on Day -1, Day 8 and Day 11 (+3 days), or ET.

ECGs will be performed after the subject rests quietly in a supine position for at least 10 min. The ECGs will be reviewed by the PI or authorized study clinician (listed on FDA Form 1572). ECGs will be analyzed for PR, QRS, and QT intervals, and for morphological abnormalities. The QT interval will be corrected by the Fridericia (QTcF) formula and recorded. To be eligible for participation, the QTcF interval must be within protocol reference range criteria at Screening and Day -1 and there must be no clinically significant ECG abnormalities. ECG PR and QTcF intervals after dosing will be reported as TEAEs if they meet toxicity grading criteria (See Appendix B, Table 4). If a question regarding ECG interpretation arises, the study investigators will have the ECG reviewed by a cardiologist.

8.2 Laboratory Evaluations

Venipuncture schedule and blood volumes are shown in Appendix A and Appendix C, respectively.

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

Blood and urine samples for clinical laboratory tests will be collected at the Screening Visit and on Day -1 to determine eligibility. For subjects eligible to enroll, measurements on Day-1 will be considered “baseline”. Blood (serum) for clinical laboratory tests (HEM, COAG, and CHEM), will also be collected on Days 2, 4 and 6 before dosing, and on Day 8 (24 h ± 1 h after last dose), and on Day 11 (+3 days), or ET. Urine for UA will also be collected on Day 4 before dosing, and

on Days 8, 11 (+3), or ET. Subjects must be fasting at least for 4 h before any blood draw for clinical laboratory assessments. These tests will include:

- HEM: Hgb, Hct, RBC, platelet count, and WBC count with absolute differential count.
- COAG: INR with prothrombin time, alpha partial thromboplastin time.
- CHEM: electrolytes (sodium, potassium, chloride, total carbon dioxide [CO_2]), creatinine with estimation of CL_{CR} by the Cockcroft-Gault method, blood urea nitrogen (BUN), calcium, glucose (fasting), total bilirubin, direct bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (AP), lactic dehydrogenase (LDH), total protein, and albumin.
- UA: Routine dipstick testing of clean-catch urine for occult blood, protein, glucose, ketones, nitrate, bilirubin, leucocyte esterase, specific gravity, and pH.
    - If urine dipstick is abnormal for blood, protein, glucose and leukocyte esterase, urine microscopy will be performed (for WBC, RBC, bacteria and other cell counts), and the results will supersede those of the dipstick UA.

Clinical laboratory tests at the Screening Visit and Day -1 should be in the normal reference range with exceptions. (See Section 5.2, Inclusion Criteria #5 and Appendix B, Table 3). Calculated CL_{CR} should be ≥80 mL/min (estimated by the Cockcroft-Gault method).

Screening laboratory values that are outside the range of eligibility but are thought to be due to an acute condition or due to collection or laboratory error may be repeated once, preferably within 24 h or as soon as subject is available.

Laboratory values will be transferred to Advantage eClinical®.

Abnormal safety laboratory values after dosing will be graded per Appendix B, Table 3. If the subject was enrolled at check-in (Day -1) with baseline values within the Grade 1 range in some laboratory tests, as allowed by the protocol (per Section 5.3.1, Inclusion Criteria #5 and Appendix B, Table 3), abnormal values in the same tests after starting study dosing will only be considered TEAEs if they deteriorate to a higher grade.

8.2.2 Viral Serology Testing

Serological testing for HIV antibody, HBsAg, and HCV antibody will be performed only at Screening Visit. These tests must be negative for study eligibility.

8.2.3 Pregnancy Testing

In all women, a serum β-HCG pregnancy test will be done at the Screening Visit and a urine pregnancy test will be done upon check in on Day -1 and results must be negative for dosing with study drug(s).

8.2.4 Serum FSH Testing

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

8.2.5 Urine Toxicology Screening

A urine toxicology screen will be performed at Screening Visit and on Day -1 to detect the presence of amphetamines, cocaine (and metabolite), barbiturates, benzodiazepines, opiates, cannabinoids (THC), tricyclic antidepressants (TCAs), and phencyclidine. Results must be negative for study eligibility. Urine creatinine will be measured as part of the profile to assess quality of collected sample.

8.2.6 Urine Alcohol and Cotinine Testing

Detection of urine alcohol and cotinine, for recent alcohol and tobacco products consumption, respectively, will be performed at the Screening Visit, and on Day -1. Results must be negative for study eligibility.

8.3 Blood and Urine Sampling and Bioanalytical Assays for Pharmacokinetics (PK)

The LC-MS/MS bioanalytical assay methods for total and free ERT and ZID quantitation in plasma and total ERT and ZID quantitation in urine will be transferred and validated at the bioanalytical lab, KCAS, before subject enrollment starts for determination of plasma and urine concentrations of ERT and ZID.

8.3.1 Blood Sampling for Plasma Total and Free ERT and ZID Assay

Blood (plasma) samples for assay of total and free ERT and ZID concentration measurement (plasma PK samples) will be collected at the following timepoints:

- On Day 1 and Day 7, samples will be collected at the following timepoints:
  - From Cohort 1 (infusion duration 0.5 h): within 30 min before dosing, and at 0.25 h (±5 min), 0.5 h (±5 min) [immediately at end of infusion], 1 h (±5 min), 2 h (±5 min), 3 h (±10 min), 4 h (±10 min), 8 h (±15 min), 12 h (±15 min), 18 h (±15 min) and 24 h (± 30 min) after the start of the infusion.
  - From Cohorts 2, 3, and 4 (infusion duration 1 h): within 30 min before dosing, and at 0.5 h (±5 min), 1 h (±5 min) [immediately at end of infusion], 2 h (±5 min), 3 h (±10 min), 4 h (±10 min), 8 h (±15 min), 12 h (±15 min), 18 h (±15 min) and 24 h (± 30 min) after the start of the infusion.

- From Cohorts 5, 6 and 7 (infusion duration 2 h): within 30 min before dosing and at 1 h (±5 min), 2 h (±5 min) [immediately at end of infusion], 3 h (±10 min), 4 h (±10 min), 8 h (±15 min), 12 h (±15 min), 18 h (±15 min) and 24 h (± 30 min) after the start of the infusion.

- On Days 2, 3, 4, 5, and 6 within 30 min before dosing and at 12 h (±15 min) after the start of the infusion.
  - **Note 1**: *On these days, the pre-dosing PK sample will be collected 24 h (± 30 min) after the start of infusion on the previous day*.
  - **Note 2:** *On Day 2, the pre-dosing PK sample can be the same as the 24 h (± 30 min) sample after the start of infusion on Day 1*.

Blood samples will be obtained promptly after each corresponding VS assessment before dosing on Days 1 to 7.

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

Details for collecting blood (plasma) samples for PK analysis and for protein binding estimation will be described in the MOP.

8.3.2 Collection of Urine for Total ERT and ZID Assay

Timed urine samples for assay of total ERT and ZID concentration measurement (urine PK samples) will be collected at the following periods:

- On Day 1, before the start of infusion, and at the following intervals after the start of the infusion: 0 to 4h (± 10 min), 4 to 8h (± 15 min), 8 to 12h (± 15 min), and 12 to 24h (± 30 min).
- On Day 7, before the start of infusion, and at the following intervals after the start of the infusion: 0 to 4h (± 10 min), 4 to 8h (± 15 min), 8 to 12h (± 15 min), and 12 to 24h (± 30 min).

8.3.3 Specimen Preparation, Handling and Shipping

8.3.3.1 Instructions for Specimens Preparation, Handling, and Storage

Plasma and urine samples collected from subjects treated with ZID, ERT or WCK (6777 ERT+ZID) need to be processed at 2°C to 8°C (35.6°F to 46.4°F) to avoid degradation. In addition, a stabilizing buffer (0.1 M MES) will be added to plasma and urine samples for total drug concentration measurements of ERT and ZID, but not to samples for free plasma concentration measurements. Details regarding the specimen preparation, handling, and storage of bioanalytical samples for the measurement of plasma total and free study drug concentration and urine total study drug concentration will be described in the protocol-specific MOP.

8.3.3.2 Specimen Shipment

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

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

Plasma (total and free) and urine (total) PK samples will be collected from all subjects and aliquots will be prepared according to protocol-specific MOP. In order to perform bioanalytical assays within the stability period of PK samples, primary aliquots of plasma and urine PK samples designated by the SDCC (Emmes) picklist will be shipped to the bioanalytical lab, KCAS, Inc., directly from the CTU within 3-4 business days after Day 8 collection in each cohort and back-up samples will be shipped after Day 11 to the DMID CMS for storage. Details will be provided in the study-specific MOP. Methods for maintaining the blind at the bioanalytical lab will be described in the Statistical Analysis Plan (SAP).

Primary aliquots of plasma and urine samples will be provided by the CTU to the bioanalytical lab, KCAS Inc., at:

Test Materials Management (TMM)
KCAS Bioanalytical and Biomarker Services
10830 South Clay Blair Boulevard
Olathe, KS, 66061
Office: 913-248-3006
Fax: 913-248-3106
Email: TMM@kcasbio.com

Back-up aliquots of plasma and urine PK samples for storage will be shipped from the CTU to DMID-CMS at:

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

Further information will be provided in the study-specific MOP.

8.4 Disposition of Plasma and Urine Samples

Blood (plasma or serum) and urine samples collected for clinical laboratory testing and PK assays will not be used for purposes other than those described. No genetic testing will be done on collected blood samples.

Clinical laboratory samples (Table 5) will be destroyed after tests are completed and results are reviewed according to standard practices of the Site Clinical Lab.

Plasma and urine PK samples used for measurement of the concentration of study drugs at KCAS, the bioanalytical lab, will be disposed of per sponsor (DMID) guidance.

9 ASSESSMENT OF SAFETY

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

Responsibilities:

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

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

9.1 Specification of Safety Parameters

Safety will be assessed by the timing, frequency, causality, and severity of:

1. Systemic and infusion site TEAEs and SAEs occurring from time of first dose with study drug(s) through Final Visit (Day 11 + 3 days), or ET.
2. Clinical laboratory TEAEs occurring from time of first dose with study drug(s) through Final Visit (Day 11 + 3 days), or ET.
3. 12-lead standard ECGs performed on Day 8 and on the Final Visit (Day 11 + 3 days), or ET.
4. Physical examinations (PE) and VS measurements performed from time of first dose with study drug(s) through Final Visit (Day 11 + 3 days), or ET.

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

9.2.1 Adverse Events

Definitions

ICH E6 defines an AE as any untoward medical occurrence in a subject or clinical investigation subject who was administered a pharmaceutical product, regardless of its causal relationship to the product. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with use of the product, and are described as treatment-emergent AEs (TEAEs). The occurrence of a TEAE may come to the

attention of study personnel during study visits and interviews, or by a subject presenting for medical care.

Expected safety information for ERT and ZID are described in detail in the product information for Ertapenem Sodium for Injection [28, 37] and the IB [34]. The severity and frequency of events listed in these sources and in the ERT product information will be taken into account for both the assessment of causality of AEs, as well as expectedness of any SAEs.

Any systemic medical condition, and the last VS and ECG measurement and clinical safety lab test value that are recorded prior to dosing on Day 1 will be considered a baseline finding for the purpose of data analysis. Baseline comprises PE, lab and ECG assessments performed on Day -1 and VS and focused PE performed on Day 1 prior to initiation of IV infusion of study drug. However, if the condition increases in severity or frequency after study drug administration at any time during the trial, it will be recorded as a TEAE.

Any medical condition that is reported after screening but before study drug administration will be evaluated and reported as MH update.

All TEAEs will be graded for severity according to Appendix B, Table 3 and Table 4 and for relationship to the study drug.

9.2.2 Severity of Events

Intensity of TEAEs will be graded as follows, unless otherwise specified in Appendix B:

Mild (Grade 1): Require minimal or no treatment; do not interfere with the subject's daily activities.

Moderate (Grade 2): Result in a low level of inconvenience or concern with therapeutic measures; may cause some interference with functioning.

Severe (Grade 3): Interrupt a subject's usual daily activity and may require systemic drug therapy or other treatment; are usually incapacitating.

9.2.3 Relationship to Study Products

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

- **Related:** There is a reasonable possibility that the study drug caused the treatment-emergent AE/SAE.
- **Not Related**: There is not a reasonable possibility that the study drug caused the treatment-emergent AE/SAE.

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

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

9.2.4 Reporting Adverse Events

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

All AEs will be documented from the time of starting study drug administration through the time of Final Visit (Day 11 + 3 days), or ET. All AEs including abnormal safety laboratory test results will be followed to resolution or until considered stable in the clinical judgment of the study investigator. Evaluation of AEs may require unscheduled visits and clinical and laboratory investigations, according to the clinical judgment of the Site PI or authorized study clinicians (listed on FDA Form 1572).

9.2.5 Serious Adverse Events

A SAE is any treatment-emergent AE that meets at least one of the following criteria:

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

  *A treatment-emergent SAE is considered "life-threatening" if, in the view of either the investigator or DMID, its occurrence places the subject at immediate risk of death. It does not include a TEAE that, had it occurred in a more severe form, might have caused death.

SAEs will be:

- Assessed for severity and relationship to study product and alternate etiology by an authorized study physician (listed on FDA Form 1572).

- Recorded on the appropriate SAE eCRF.
- Followed through resolution.
- Reviewed and evaluated by the SMC (periodic review unless related), DMID, and the IRB.

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

A licensed study clinician (listed on FDA Form 1572) will determine seriousness, severity, and causality of abnormal laboratory values; provide a medical evaluation of TEAEs; and classify TEAEs as clinically significant (CS) or not clinically significant (NCS) based upon medical judgment.

Abnormal laboratory values or clinical findings for all enrolled subjects after dosing will be assessed using the toxicity scales in Appendix B, Table 3. Abnormal values assessed as Grade 1 in severity and noted at screening or baseline but allowed for enrollment per Section 5.1, Inclusion Criteria #5 will only be considered TEAEs if they increase in severity to Grade 2 or higher. For abnormalities noted from the time of study drug dosing, any Grade 1 or higher laboratory abnormality listed on the toxicity in Appendix B, Table 3will be entered in the database as a TEAE. Gross blood in urine that is confirmed due to menses is not a TEAE (but is for all other reasons). Abnormal laboratory values, performed as part of HEM, COAG, CHEM, or UA but not listed in this toxicity table will be evaluated by the study clinicians, recorded in the source document and, if clinically significant, considered TEAEs and graded according to the criteria in Section 9.2.1.

Protocol-specific laboratory normal range values in effect at the time of protocol submission for evaluation of TEAEs are included in Appendix B, Table 3.

9.3 Reporting Procedures

9.3.1 Serious Adverse Events

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

All SAEs will be:

- Recorded on the appropriate SAE report form and sent to DMID Pharmacovigilance Group (PVG).
- Entered into the appropriate subject source document and eCRF in Advantage eClinical®.
- Reported to the IRB.
- Reviewed and followed to resolution or stability by an authorized study physician (listed on FDA Form 1572).

- Collected on each subject until Day 11 + 3 days (Final Visit), or ET Visit.

Any AE that meets a protocol-defined serious criterion will be submitted immediately (within 24 h of site awareness) on an SAE report form to DMID PVG 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 report form, selected SAE data fields will also be entered into Advantage eClinical®. Please see the protocol-specific MOP for details regarding this procedure.

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

The DMID Medical Monitor (MM) and DMID CPM will be notified of the SAE by the DMID PVG. The DMID MM will review and assess the SAE for regulatory reporting and potential impact on subject safety and protocol conduct.

At any time after completion of the trial, if the Site PI or authorized study clinician (listed on FDA Form 1572) becomes aware of an SAE that is suspected to be related to study drug, the Site PI or authorized study clinician will report the event to the DMID PVG.

9.3.2 SUSAR Regulatory Reporting for Studies Conducted under DMID Sponsored IND

Following notification from the investigator, DMID, the Investigational New Drug (IND) sponsor, is responsible for making the determination of which SAEs are Suspected unexpected serious adverse reactions or SUSARs. A SUSAR, as defined in 21 CFR 312.32, is any SAE where a causal relationship with the study product is at least reasonably possible but that adverse event is not listed in the IB, Package Insert, and/or Summary of Product Characteristics.

All SUSARs will be reported to the FDA as IND Safety Reports per 21 CFR 312.32 as soon as possible but not exceeding 7 calendar days for unexpected fatal or life-threatening events, and not exceeding 15 calendar days for other qualifying events. IND Safety Reports will also be provided to the IRB.

The DMID Medical Monitor and Clinical Project Manager will be notified of the SAE and potential SUSAR by the DMID Pharmacovigilance Group. The DMID Medical Monitor will

review and assess SUSARs for regulatory reporting and potential impact on study subjects' safety and protocol conduct.

DMID will submit an IND safety report to the FDA and will notify all participating site Principal Investigators of potential serious risks from clinical studies or any other source, as soon as possible.

All SAEs designated as "not related" to study product(s), will be reported to the FDA and to the national regulatory authorities at least annually in a summary format (e.g., IND Annual Report).

9.3.3 Other Adverse Events (if applicable)

9.3.3.1 Reporting of Overdose

An overdose is defined as a dose greater than the high-dose level evaluated in the trial as described in Section 6.3.1. All overdoses will be reported; if the overdose is associated with a TEAE, then the TEAE will also be reported. In the event of an overdose of study drug, the investigator will use clinical judgment in treating the overdose and contact the DMID MM. There is no specific antidote in case of ERT or ZID or WCK 6777 (ERT plus ZID) overdose. The same measures as recommended for other antibiotics belonging to the class of carbapenem antibiotics (treatment cessation, supportive treatment, adequate hydration, and hemodialysis in case of severe overdosage) should be considered until further clinical data are available for the study drug(s). The investigator will refer to the relevant document(s) for detailed information regarding warnings, precautions, contraindications, TEAEs, and other significant data pertaining to each study drug. Such documentation may include but not be limited to Prescription Information for Ertapenem Sodium for Injection (ERT) (28, 37), and the 2022 WCK 6777 Investigator Brochure (34).

9.3.3.2 Reporting of Pregnancy

Pregnancies that occur in female subjects during the trial will be reported via Advantage eClinical® on a pregnancy report form. With the subject's permission, all protocol-required venous blood samples will be obtained, and the subject will continue to be followed for safety until Day 11 + 3 days (Final Visit). Efforts will be made to follow all pregnancies reported during the trial to pregnancy outcome, as described in the protocol-specific MOP (e.g., delivery, spontaneous abortion, or therapeutic abortion), pending the subject's permission.

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

Serious adverse outcomes of pregnancy affecting the mother and/or fetus or neonate (e.g., spontaneous abortion, congenital anomaly(ies) in fetus or child, late fetal death, or reports of adverse drug reactions in a newborn/neonate that is fatal, life-threatening, resulting in persistent or

significant disability/incapacity or resulting in or prolonging hospitalization) will be documented in a SAE form and reported to the CROMS PVG within 24 hours of clinical site awareness of the events.

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

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

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

9.5 Halting Rules

9.5.1 Study Halting Criteria

- One subject experiences an SAE regardless of the relationship to study product, with the exception of accidental injury. from the time of first dose through the last visit (Day 11 +3).
- Two or more subjects, in the same cohort, experience Grade 3 (severe) AEs (Systemic or Laboratory) through the last visit (Day 11+3) regardless of the relationship to study product that are coded in the same High Level Term (HLT) per MedDRA classification.
- In combination treatment cohorts (Cohorts 1, 4 and 7), three or more subjects (cumulative) experience Grade 3 (severe) AEs (Systemic or Laboratory) regardless of the relationship to study product with the exception of accidental injury that are coded in the same High Level Group Term (HLGT) per MedDRA classification.
    - **Note:** For Grade 3 TEAEs in the situations above, VS abnormalities will be considered part of a systemic disorder or an organ-specific condition, as described in Appendix B: Adverse Events Toxicity Grading Criteria, in order to be included among the Study Halting Criteria.

If the predefined criteria for the halting rules are met, the SMC will review the study data and provide guidance on how to proceed.

A halted study will resume upon recommendation by the Sponsor (DMID).

9.5.2 Individual Subject Halting Criteria

A subject will be discontinued from further dosing if any of the following criteria are met:

- A subject experiences a SAE, regardless of the relationship to the study product.
- A subject develops anaphylaxis within 24 hours after receiving the study product(s).

- A subject experiences a suspected drug-related hypersensitivity AE Grade 2 or higher during the infusion. In that case, the infusion must be stopped, and that subject will not continue with dosing.
- A subject experiences a Grade 3 AE event (laboratory or systemic) if not resolved within 24 hours of dosing.
- If in the opinion of the site PI or authorized study clinician (listed on FDA Form 1572), further dosing would not be in the best interest of the subject.

9.5.3 Dose Escalation Halting Criteria

Administration of drug product(s) in the next higher dose cohort should not occur unless all participants in the previous dose cohort(s) have received the study products and safety data from those participants are reviewed in accordance with the protocol (See Section 4 and Section 9.6.1).

For dose escalation from the combination Cohort 1 (1 g ERT + 1 g ZID) to higher single dose Cohorts 2 (2 g ERT) and 3 (2 g ZID), and from the combination Cohort 4 (2 g ERT + 2 g ZID) to higher single dose Cohorts 5 (3 g ERT) and 6 (3 g ZID), the study will proceed to the next planned dose level upon recommendation by the SMC following review of cumulative interim safety data after completion of Cohort 1 or Cohort 4, respectively. The recommendation to continue to the next planned combination treatment cohorts (i.e., Cohort 4 and Cohort 7 [3 g ERT +3 g ZID]) will be made by the study team (DMID MO and MM and the site PI) after review of blinded safety data in Cohorts 2 and 3 and Cohorts 5 and 6, respectively. For dose escalation to single or combination cohorts, the SDCC confirms and notifies the SMC and/or study team (DMID MM, DMID MO and study PI) as needed that none of the following criteria were met:

- Three or more subjects in the same cohort experience an AE, grade 2 or higher (Systemic or Laboratory), regardless of the relationship to study the study product with the exception of accidental injury in the same system organ Preferred Term (PT) per MedDRA classification.
- Two or more subjects in the same cohort experience at least one Grade 3 AE and at least one Grade 2 AE, regardless of the relationship to study product with the exception of accidental injury, in the same HLT per MedDRA classification.

If any of the above criteria is met, escalation to the next planned dose cohort will not proceed until all available study data have been reviewed by the SMC. If, following review by the SMC, this is deemed acceptable to restart the study, the study will resume with DMID (sponsor) agreement.

9.6 Safety Oversight

9.6.1 Safety Monitoring Committee (SMC)

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

The SMC will meet as follows:

- Organizational meeting (before study initiation).
- *Ad hoc* meeting.
  - When study halting criteria are met following review by the study team (DMID MO and MM and the site PI).
  - At the request of DMID to review a potential safety concern identified by either the Site PI or DMID MM.
- *Scheduled* meeting.
  - The SMC will review cumulative interim safety data after completion of all subjects in Cohort 1.
  - The SMC will review cumulative, interim safety data when available after completion of all subjects in Cohorts 1 to 4.
  - The SMC will review final safety data in all cohorts when available after database lock.

Procedures for SMC reviews/meetings will be defined in the SMC charter. The SMC will review safety data and provide recommendations to DMID, NIAID on resumption or stopping of a temporary halted trial.

10 CLINICAL MONITORING

10.1 Site Monitoring Plan

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

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

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

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

11 STATISTICAL CONSIDERATIONS

11.1 Study Hypotheses

The objectives of the study are to obtain safety data and plasma and urine PK data for 3 escalating doses of WCK 6777 (combination of ERT with ZID) and 2 escalating doses of ERT alone and ZID alone. There are no formal hypotheses being tested in this Phase 1 trial.

11.2 Sample Size Considerations

The sample size of 52 subjects assigned into 7 treatment cohorts with 8 subjects per cohort (6 subjects randomized to receive active drug(s) and 2 placebo), with the exception of Cohorts 3 and 6, where 6 subjects will receive active ZID only, was chosen to obtain reasonable evidence of safety without exposing undue numbers of healthy subjects to ERT combined with ZID, ERT alone, and ZID alone at this phase of clinical evaluation. Previous experience in Phase 1 studies has shown that the sample size being proposed is sufficient to fulfill the primary and secondary objectives of the study. Power calculations have not been performed.

It is expected there will be 7 evaluable subjects in each cohort with 8 subjects each (ERT standalone Cohorts 2 and 5 and WCK 6777 cohorts 1, 4 and 7), and 5 subjects in each ZID standalone cohort (Cohorts 3 and 6).

11.3 Safety Review

An SMC will be appointed to oversee the safe conduct of the trial. A scheduled SMC meeting will be held to review safety data from Cohorts 1 to 4 and decide on continuation of the study to Cohorts 5 to 7. If the study is continued, a final SMC meeting will be held to review cumulative data and to make recommendations about the safety profile and safety monitoring of the clinical trial. The decision to continue the study after Cohort 1, Cohorts 2 and 3, and Cohorts 5 and 6 will be made by DMID based on review of safety data and summary provided by the site PI. If criteria for halting the trial (as listed in Section 9.5.1) are met, an *ad hoc* SMC meeting will be held to review all available safety data and to make recommendations about the dosing of all further subjects in the trial. Blinded data will be presented at the open SMC meeting. An unblinded statistician may present unblinded data in a closed session of an SMC meeting.

11.4 Final Analysis Plan

The ICH Guidance E9 (Statistical Principles for Clinical Trials) will be followed for all statistical content. Summaries of frequencies and percentages will be presented for categorical data, and summary statistics such as median, mean, standard deviation (SD), minimum and maximum values will be presented for continuous data by Dose Cohort. Details of the statistical methods and models used for plasma and urine PK analyses, including dose proportionality, and safety data analyses and presentations will be described in the Statistical Analysis Plan (SAP) and

accompanying Tables, Listings and Figures (TLF) templates. The SAP will be prepared and finalized by the SDCC before analysis of the safety and PK data. A final analysis containing safety data and PK data from all study cohorts will be performed by the SDCC after final data lock and included in the clinical study report (CSR). Any change from originally planned statistical analyses will be reported in the CSR.

11.4.1 Analysis Populations

The *safety population* will include all subjects who received any amount of study drug(s).

The *PK analysis population* will consist of all subjects who received at least a single dose of study drug and have at least one quantifiable post-dosing plasma drug concentration measured. The *PK Analysis Subset* is defined as the subset of the PK Analysis Population that received the intended doses of study drug(s) and have adequate data that permit estimation of PK parameters. For the PK analysis and estimates of exposure parameters, a subject will be considered to have a complete PK profile if ≥70% of plasma PK samples are collected. For participants who have incomplete PK profiles, their available PK data will be considered in the PK analyses. Subjects will be analyzed by dose as treated.

If any subjects are found to be noncompliant with respect to dosing or have incomplete data, a decision to include them in the analysis will be made by criteria that would be described in the Statistical Analysis Plan.

11.4.2 Demographics and Baseline Characteristics

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

11.4.3 Safety Analysis

11.4.3.1 Adverse and Serious Adverse Events

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

- Overall (i.e., regardless of severity or relationship to treatment).
- By severity grade (mild, moderate, or severe).
- By relationship to study drug.

- By MedDRA level hierarchy (system organ class [SOC], higher level group term [HLGT] and preferred term [PT]).

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

11.4.3.2 Additional Safety Analyses

Descriptive summary statistics (mean, SD, median, minimum, and maximum) for all clinical laboratory data, 12-lead standard ECG parameters, and VS at admission and each applicable post-dosing visit, including changes from baseline values, will be calculated and presented by treatment cohort. Baseline values will be the last values recorded before administration of study drug. For change-from-baseline summaries, subjects with an undefined change from baseline, due to missing data, will be excluded. Clinical significance of abnormalities, as assessed by the study PI or authorized study clinicians, will be indicated. The number and percentage of subjects who meet toxicity criteria for clinical laboratory, VS and 12-lead ECG investigations will be listed and tabulated by treatment cohort.

11.4.3.3 Tolerability Assessment

Tolerability to study product will be summarized by dose cohort and include number and incidence of subjects who: 1) discontinued study drug due to an AE, 2) chose to withdraw from the study due to an AE at any time from start of dosing until end of study, or 3) were withdrawn from the study by the investigator due to an adverse event at any time from start of dosing until end of study.

11.4.4 PK Analysis

Plasma (total and free) and urine concentrations of ERT and ZID on Day 1 and Day 7 will be summarized using descriptive statistics (n, mean, SD, CV%, median, minimum, and maximum) for each treatment cohort, study day and nominal timepoint. Plasma (total and free) study drug concentrations observed before dosing on Days 2, 3, 4, 5, 6 and 7 and 24 h after dosing on Day 7 (trough concentrations) and 12 h (± 15 min) after start of infusion on Days 1 to 7 will be summarized similarly for each treatment cohort and study day.

Plasma (total and free) and urine PK parameters will be estimated separately for ERT and ZID by noncompartmental analysis (NCA) methods using Phoenix® WinNonlin® version 8.0 or higher and presented by treatment cohort. PK parameters and linearity index and dose-normalized exposure parameters ($AUC_{(0\text{-}tau)}$/Dose and C_{max}/Dose) after the first dose and multiple doses will be analyzed and presented.

Graphical presentations of plasma concentration vs. time profiles will be provided for ERT and ZID and will include individual subject and mean concentration profiles. Semi-log concentration

profiles will be provided for individual subjects. Graphical presentation of mean and individual trough study drug concentrations and study drug concentrations collected 12 h after dosing through Day 7 will be displayed.

% Protein binding for ERT and ZID will be calculated and presented by treatment cohort at all collected timepoints the results will be tabulated and presented graphically.

11.4.5 Plasma PK parameters:

The study population and methodology used to evaluate individual agent and combination PK parameters will be described in detail in the PK Plan and is reviewed briefly below.

The following *single-dose plasma PK parameters* will be computed (if estimable) from the plasma total and free concentration-time data over the 24-hour following Dose 1 for each study drug:

- C_{max}: observed maximum concentration
- T_{max}: Time of maximum concentration (h)
- C_{min}: observed minimum concentration at the end of the dosing interval
- $AUC_{(0\text{-}t)}$: area under the plasma concentration -time curve to the last time with a concentration greater than or equal to the validated limit of quantitation of the assay
- $AUC_{(0\text{-}\infty)}$: area under the plasma concentration-time curve to infinity
- $AUC_{(0\text{-}24)}$: area under the plasma concentration - time curve extrapolated to 24 h after dosing
- $AUC_{(0\text{-}last)}$: Area under the plasma concentration - time curve from time zero to the last concentration above the lower limit of quantitation
- $AUC_{(0\text{-}tau)}$: area under the plasma concentration - time curve to the end of the dosing interval
- $t_{1/2}$: Terminal half-life
- CL_T: Total clearance
- K_e: terminal phase elimination rate constant
- Vd: Volume of distribution
- Dose-normalized exposure parameters ($AUC_{(0\text{-}tau)}$/Dose and C_{max}/Dose)

The following *multiple-dose plasma PK parameters* will be computed (if estimable) from the drug total and free concentration-time data over the 24-hour dosing interval following Dose 7 for each study drug.

- $C_{max,ss}$: observed maximum concentration at steady state
- $C_{min,ss}$: observed minimum concentration at the end of the dosing interval at steady state
- C_{avg}: calculated average concentration during the dosing interval
- $T_{max,ss}$: Time of maximum concentration (C_{max}) at steady state

- T_{min}: Time to minimum concentration (C_{min})
- $AUC_{(0\text{-}24),ss:}$ area under the plasma concentration - time curve extrapolated to 24 h after dosing
- $AUC_{(0\text{-}tau),ss}$: Area under the plasma concentration - time curve to the end of the dosing interval at steady state
- $t_{1/2}$: Terminal half-life (Day 7 only)
- CL_T: Total clearance
- $V_{d,ss}$: Volume of distribution at steady state
- Linearity Index: $AUC_{(0\text{-}tau),ss}$ (Day 7) / $AUC_{(0\text{-}\infty)}$ (Day 1)
- RAUC : Accumulation ratio for AUC estimated as $AUC_{(0\text{-}tau)}$) (Day 7) / $AUC_{(0\text{-}24)}$ (Day 1)
- RC_{max}: Accumulation ratio for C_{max} estimated as C_{max} (Day 7) / C_{max} (Day 1).
- Dose-normalized exposure parameters ($AUC_{(0\text{-}tau)}$/Dose and C_{max}/Dose)

11.4.6 Urine PK parameters:

The results of urine PK parameters for each individual study drug in each cohort will be listed by subject at each dose, and summarized with descriptive statistics (n, mean, SD, coefficient of variation, median, minimum, maximum, geometric mean and geometric SD) at each collection period. When evaluable, estimated urine PK parameters will include:

- Ae,urine: Amount of unchanged drug excreted in urine during each collection interval following Dose 1 and Dose 7 (i.e., at 0-4, 4-8, 8-12, 12-24 h after initiation of infusion)
- $Ae,urine_{(0\text{-}24)}$: Cumulative amount of unchanged drug excreted in urine from zero (predose) to 24 h following Dose 1
- $Ae,urine_{(0\text{-}24),ss}$: Cumulative amount of unchanged drug excreted in urine from zero (predose) to 24 h after Dose 7
- fe,urine: Fraction (%) of dose excreted unchanged in urine during each collection interval following Dose 1 and Dose 7 (i.e., at 0-4, 4-8, 8-12, 12-24 h after initiation of infusion)
- $fe,urine_{(0\text{-}24)}$: Fraction (%) of dose excreted unchanged in urine from zero (predose) to 24 h after Dose 1
- $fe,urine_{(0\text{-}24),ss}$: Fraction (%) of dose excreted unchanged in urine from zero (predose) to 24 h following Dose 7
- $CL_{r(0\text{-}24)}$: Renal clearance (L/h) following Dose 1
- $CL_{r,ss}$: Renal clearance following Dose 7

11.4.7 Missing Values and Outliers

All attempts will be made to collect all data per protocol. No imputation will be performed for missing values. Rules for identifying outliers will be described in the SAP. Outliers will not be

excluded from the primary analyses. Outliers identified during the PK analysis will be discussed in the analysis report.

11.4.8 Multiple Comparisons / Multiplicity

This is a small Phase 1 Study with multiple endpoints. Because analyses of endpoints are descriptive rather than hypothesis tests, no adjustments for multiplicity testing are planned.

11.4.9 Interim Reports

Interim cumulative safety data will be presented to the SMC after completion of Cohort 1 and Cohort 4.

Blinded safety and tolerability data will be periodically reviewed by DMID and the site PI or authorized study clinician (listed on FDA Form 1572) to assess whether rules for halting progression to the next study cohort have been met. An *ad hoc* SMC meeting will review safety data if halting criteria have been met. The SMC will receive data in aggregate. The SMC may request to receive data by study product vs. placebo in a closed session. The SMC may also request that the blind be broken for individual subjects, as needed, to assess safety issues.

12 SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA/DOCUMENTS

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

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

13 QUALITY CONTROL AND QUALITY ASSURANCE

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

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

14 ETHICS/PROTECTION OF HUMAN SUBJECTS

14.1 Ethical Standard

The 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 25692 (1997), if applicable. The Site PI's institution will hold a current Federal Wide Assurance (FWA) issued by the Office of Human Research Protection for federally funded research.

14.2 Institutional Review Board

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

14.3 Informed Consent Process

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

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

Study staff will inform subjects that the trial involves research, and explain the purpose of the trial, those aspects of the trial that are experimental, any expected benefits, all possible risks (including a statement that the particular treatment or procedure may involve risks to the subject or to the embryo or fetus, if the subject is or may become pregnant, that are currently unforeseeable), the expected duration of the subject's participation in the trial, the procedures of the research study, including all invasive procedures, and the probability for assignment to treatment cohorts. Subjects will be informed that they will be notified in a timely manner if information becomes available that may be relevant to their willingness to continue participation in the trial. They will also be

informed of alternative procedures that may be available, and the important potential benefits and risks of these available alternative procedures. Subjects will receive an explanation as to whether any compensation and any medical treatments are available if injury occurs, and, if so, what they consist of, or where further information may be obtained. Subjects will be informed of the

anticipated financial expenses, if any, to the subject for participating in the trial, as well as any anticipated prorated payments, if any, to the subject for participating in the trial. They will be informed of whom to contact (e.g., the Investigator) for answers to any questions relating to the research project. Information will also include the foreseeable circumstances and/or reasons under which the subject's participation in the trial may be terminated. Subjects will be informed that participation is voluntary and that they are free to withdraw from the trial for any reason at any time without penalty or loss of benefits to which the subject is otherwise entitled.

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

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

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

Children aged < 18 years will be excluded from participation because insufficient data are available in adults to judge potential risk in children, and as a Phase 1 trial, there is no known benefit.

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

Women of childbearing potential will be included but will be repeatedly counseled to use effective measures (Section 5.1) to avoid becoming pregnant from the time of screening to 30 days following the last dose of study product, because the effects of the study drugs on the unborn fetus are not known.

14.5 Subject Confidentiality

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

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

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

14.6 Certificate of Confidentiality

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

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

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

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

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

14.7 Study Discontinuation

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

- Incidence or severity of TEAEs indicates a potential health hazard.
- Data recording is inaccurate or incomplete.
- Investigator does not adhere to the protocol or applicable regulatory guidelines in conducting the trial.

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

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15 DATA HANDLING AND RECORD KEEPING

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

Data collection forms will be provided by the SDCC and the CTU will use them to develop the CTU's source documents to record and maintain data for each subject enrolled in the trial. Data reported in the eCRF derived from source documents will be consistent with the source documents or the discrepancies will be explained.

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

15.1 Data Management Responsibilities

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

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

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

15.2 Data Capture Methods

Clinical data (including, but not limited to treatment-emergent AE/SAEs, ConMeds, MH, PE, clinical laboratory data, and ECG data [ECG intervals and interpretations]) will be collected on data collection forms by study personnel then entered into eCRFs via a 21 CFR 11-compliant Internet Data Entry System provided by the SDCC. The data system includes password protection and internal quality checks (e.g., automatic range checks) to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the source documents by CTU study personnel.

15.3 Types of Data

Data for the trial will include clinical and laboratory safety assessments, 12-lead standard ECG interval measurements and interpretations, plasma and urine study drug concentrations at each specified timepoint and PK parameters for each dose of individual study drugs (ERT and ZID) and of WCK 6777 (combination ERT and ZID).

15.4 Timing/Reports

A final CSR will be prepared after all safety, 12-lead standard ECG, and plasma and urine PK data are available.

15.5 Study Records Retention

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

15.6 Protocol Deviations

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

These practices are consistent with ICH E6:

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

5.1 Quality Assurance and Quality Control, Section 5.1.1

5.20 Noncompliance, Sections 5.20.1, and 5.20.2.

It is the responsibility of the Site PI/study staff to use continuous vigilance to identify and report deviations within 5 working days of identification of the protocol deviation, or within 5 working days of the scheduled protocol-required activity. All deviations will be promptly reported to DMID via SDCC's Advantage eClinical®.

All protocol deviations, as defined above, will be addressed in 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 source document. Protocol deviations will be sent to the local IRB/EC per their guidelines. The Site PI/study staff is responsible for knowing and adhering to their IRB requirements.

16 PUBLICATION POLICY

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

Refer to:

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

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

Refer to:

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

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18 APPENDICES

APPENDIX A: SCHEDULE OF STUDY PROCEDURES AND EVALUATIONS

Evaluation (↓)	Screening [1]	Check-in/ Enrollment	In-patient Treatment								Out-patient follow-up	Unscheduled / Early Termination
Visit (V) ->	1	2									3	
Study Day (D) ->	-28 to -2	-1	1	2	3	4	5	6	7	8	11 (+3)	
Informed consent	X											
Confirmation of eligibility criteria	X	X [2]	X [2]									
Medical History	X											
Demographics	X											
Height, Weight and BMI [3]	X											
Weight		X [2]								X		
MH update		X [2]	X [2]									
Prior and Concomitant medications [4]	X	X	X [4]	X [4]	X [4]	X [4]	X [4]	X [4]	X [4]	X [4]	X [4]	X [4]
Physical examination (PE)	X	X								X	X	X
Symptom directed (focused) PE [5]			X	X	X	X	X	X	X			
Vital signs [6]	X	X	X	X	X	X	X	X	X	X	X	X
ECG [7]	X	X								X	X	X
Clinical Laboratory testing (HEM, CHEM, and COAG) [8]	X	X		X		X		X		X	X	X
Urinalysis [9]	X	X				X				X	X	X
Viral serology [10]	X											
Serum HCG pregnancy test (all females) and FSH (post-menopausal females only) [11]	X											
Urine pregnancy test (all females) [11]		X										
Urine drug screen [12]	X	X										
Urine alcohol test	X	X										
Urine cotinine test	X	X										
Admission of eligible subjects /Assignment to treatment cohort / Randomization [13]		X										
Study drug administration [14]			X	X	X	X	X	X	X			
Assessment of adverse events [15]			X	X	X	X	X	X	X	X	X	X
Assessment of infusion and blood drawing sites [16]			X	X	X	X	X	X	X	X	X	X
Plasma PK [17]			X [17]	X [17]	X [17]	X [17]	X [17]	X [17]	X [17]	X [17]		X [18]
Urine PK [19]			X [19]						X [19]			
Discharge from in-patient site										X		
Final visit / Discharge from study											X	
Counseling	X	X	X	X	X	X	X	X	X	X	X	X

1. Screening evaluations must be completed within 28 days prior to administration of study drug(s).
2. Confirmation of eligibility for admission and enrollment and randomization on Day -1, and for dosing on Day 1 after completion of all baseline procedures before study intervention
   - Baselines are: Complete PE, weight, Clinical labs and ECG on Day-1; VS (within 30 min before dosing on Day 1), MH and Prior Medications updates, and Focused PE (as needed for new symptoms) predose on Day 1
3. BMI ≥18-32 kg/m^2 and Weight ≥ 100 lbs (At Screening). Weight also measured on Day 8.
4. Concomitant medications include any new medications taken only after initiation of first dose on Day 1.
5. Symptoms directed (focused) PE for evaluation of new symptoms prior to dosing on Day 1, and for evaluation of TEAEs after dosing on Day 1 to the end of the study, or ET
6. Vital Signs: collected after 5 minutes of resting in supine position and include SBP, DBP, HR and T (oral measurement). Recorded at Screening, Day -1, Days 1 to 7 (within 30 min prior to dosing, at the end of infusion (+10 min) and 1 hour (±5 min) after the end of infusion), Day 8, and Day 11 (+3), or ET.
7. Subjects must be lying supine or sitting for 10 minutes prior to ECG. ECG will be done in triplicate 2 minutes apart at Screening only. Single ECGs will be recorded on Days -1, 8, and 11 (+3).
8. Clinical Laboratory testing for Hematology (HEM: Hemoglobin, hematocrit, RBC, WBC with WBC differential count, and platelet count), Clinical Chemistry (CHEM: sodium, potassium, chloride, bicarbonate, BUN, creatinine, estimated CLCR (by the Cockcroft-Gault method), calcium, glucose, albumin, total protein, total bilirubin, direct bilirubin, ALT, AST, ALP and LDH) and Coagulation (COAG: PT and AP). – Lab values on Day -1 are considered baseline.
9. Urinalysis: Dipstick urinalysis, including protein, glucose, ketones, bilirubin, occult blood, nitrite, leukocyte esterase, specific gravity, and pH. If dipstick UA is abnormal for blood, protein, glucose and leukocyte esterase, a microscopic UA will be done (for WBC, RBC, bacteria, and other cell counts), and results will supersede those of the dipstick UA. Lab values on Day -1 are considered baseline.
10. Viral serology: HIV antibody, HBsAg, HCV antibody
11. Serum pregnancy at Screening and Urine pregnancy test on Day-1 from all females; FSH at Screening only from post-menopausal females.
12. Urine drug screen panel for: amphetamines, barbiturates, benzodiazepines, cocaine metabolites, cannabinoids (THC), opiates, phencyclidine, and tri-cyclic antidepressants – At Screening and Day -1
13. Assignment to dosing cohort and randomization: Must occur after all inclusion and exclusion criteria have been confirmed on Day -1 and subject was admitted to the in-patient site.
14. Study drug or placebo administered IV according to cohort and randomization assignment. Withhold solid food at least 4 h before starting the infusion and 1 h after completion of infusion. Oral liquids are allowed.
15. Monitoring for treatment-emergent AEs and SAEs will begin upon initiation of the first dose of study product(s) and continue until the last visit, or ET.
16. Assess and replace IV catheters no more frequently than every 72 to 96 h or as clinically indicated.

17. Plasma PK collection times:
    DAY 1 and DAY 7:
    - For the 30 min infusion (Cohort 1): Predose (within 30 min; t=0) and at 0.25 h (±5 min), 0.5 h (±5 min) (immediately at end of infusion), 1 h (±5 min), 2 h (±5 min), 3 (±10 min), 4 h (±10 min), 8 h (±15 min), 12 h (±15 min), 18 (±15 min) and 24 h (±30 min) after start of infusion
    - For the 60 min infusion (Cohorts 2, 3 and 4): Predose (within 30 min; t=0) and at 0.5 h (±5 min), 1 h (±5 min) (immediately at end of infusion), 2 h (±5 min), 3 h (±10 min), 4 h (±10 min), 8 h (±15 min), 12 h (±15 min), 18 h (±15 min) and 24 h (±30 min) after start of infusion
    - For the 120 min infusion (Cohorts 5, 6 and 7): Predose (within 30 min; t=0) and at 1 h (±5 min), 2 h (±5 min) (immediately end of infusion), 3 h (±10 min), 4 h (±10 min), 8 h (±15 min), 12 h (±15 min), 18 (±15 min) and 24 (±30 min) after start of infusion

    DAY 2 to DAY 6:
    - Predose (within 30 min) and 12 (±5 min) h after start of infusion.
18. Unscheduled visit – plasma PK collection is optional
19. Urine PK collection times
    DAY 1 and DAY 7:
    - Before the start of infusion (within 60 min), and at the following intervals after the start of the infusion: 0 to 4 h (±10 min), 4 to 8 h (±15 min), 8 to 12 h (±15 min), and 12 to 24 h (±30 min),

APPENDIX B: ADVERSE EVENTS TOXICITY GRADING CRITERIA

ABBREVIATIONS: Abbreviations utilized in the Tables:

ADL = Activities of Daily Living
CTU = Clinical Trial Unit
ULN = Upper Limit of Normal
IV = Intravenous

ESTIMATING SEVERITY GRADE

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

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

SERIOUS OR LIFE-THREATENING AEs

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

COMMENTS REGARDING THE USE OF THESE TABLES

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

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

Table 2 Toxicity Grading Tables – CLINICAL AEs

Clinical AEs	Reference range	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
VITAL SIGNS [1]				
Fever - °C Fever - °F	36.1 - 37.2 [1] 97.0 - 99.0 [1]	37.3 - 38.4 99.1 - 101.1	38.5 - 38.9 101.2 - 102.0	>38.9 >102.0
Tachycardia - bpm	50 - 100 [2,3,4,5]	101 - 115	116 - 130	>130 or ventricular dysrhythmias
Bradycardia – bpm		45 - 49	40 - 44	<40
Hypertension (systolic) - mmHg	130/89 [2,3,4,5]	131 - 150	151 - 160	>160
Hypertension (diastolic) - mmHg		90 - 95	96 - 100	>100
Hypotension (systolic) - mmHg		85 - 88	80 - 84	<80
Tachypnea – breaths per min	10 - 20 [2,3,4,5]	21 - 25	26 - 30	>30

Note 1: No recent hot or cold beverages or smoking. A protocol should select either ^{0}C or ^{0}F for inclusion.

Note 2: Assume awake and in supine position for 5 min at rest. Abnormal HR and BP on first measurement due to a technical (procedural) error may be repeated twice more with the subject resting between measurements for at least 5 min (See Section 8.1.6 Vital Signs).

Note 3: Exceptions to screening BP and HR reference range are:

(a) Subjects with baseline HR ≥ 45 to 50 bpm may be accepted if otherwise healthy adults with known history of asymptomatic bradycardia.

(b) Subjects with baseline SBP up to 140 mmHg and DBP up to 90 mmHg may be accepted if otherwise healthy.

Note 4: Isolated/individual abnormalities of VS would not be considered toward halting criteria. Abnormalities of VS will be described as “increased X” or “decreased X” (X = HR, BP, RR, temperature) if asymptomatic, transient and not associated with a systemic or organ-specific disorder and coded by MedDRA within the System Organ Class (SOC) “Investigations.” These abnormalities will be graded per criteria in this table, but not considered in determining whether study stopping criteria have been met. On the other hand, abnormalities of VS that are either secondary to systemic or organ-specific clinical syndrome or primary disorders will be coded in the appropriate SOC (e.g., “cardiac disorders”, “respiratory disorders”, “immunological disorders”, etc.). These abnormalities will be considered in determining whether stopping criteria have been met.

Table 2 Toxicity Grading Tables – CLINICAL AEs

Clinical AEs	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
CARDIOVASCULAR DISORDERS			
Arrhythmia (except Sinus tachycardia)		Asymptomatic or transient signs; no medical intervention required.	Recurrent and/or persistent signs; symptomatic medical intervention required.
Hemorrhage	Estimated blood loss ≤100 mL.	Estimated blood loss >100 mL; no transfusion required.	Blood transfusion required.
RESPIRATORY DISORDERS			
Cough	Transient cough; no treatment required.	Persistent cough; treatment required.	Interferes with daily activities.
Bronchospasm, Acute	Transient bronchospasm; no treatment required; FEV1 71-80% of predicted peak flow.	Requires treatment; normalizes with bronchodilator; FEV1 60-70% of predicted peak flow.	No normalization with bronchodilator; FEV1 <60% of predicted peak flow.
Dyspnea	Does not interfere with usual and social activities.	Interferes with usual and social activities; no treatment.	Prevents usual and social activities OR requires treatment.
EAR AND LABYRINTH DISORDERS			
Tinnitus	Mild symptoms: intervention not indicated.	Moderate symptoms; limiting instrumental ADL.	Severe symptoms; limiting self-care ADL.
Vertigo	Mild symptoms.	Moderate symptoms; limiting instrumental ADL.	Severe symptoms; limiting self-care ADL.
GASTROINTESTINAL DISORDERS			
Nausea	No interference with normal activity.	Some interference with normal activity.	Prevents daily activities.
Vomiting	No interference with activity OR 1-2 episodes in a 24-h period.	Some interference with activity OR >2 episodes in a 24-h period.	Prevents daily activity OR requires medical intervention.
Diarrhea	2-3 loose OR watery stools in a 24-h period.	4-5 loose OR watery stools in a 24-h period.	6 or more loose or watery stools in a 24-h period OR requires IV hydration OR requires medical intervention.
Oral Discomfort / Dysphagia	Mild discomfort; no difficulty swallowing.	Some limits on eating /drinking.	Eating/talking very limited; unable to swallow solid foods.
NEUROLOGICAL DISORDERS			
Dizziness	Mild unsteadiness or sensation of movement	Moderate unsteadiness or sensation of movement; limiting instrumental activities of daily life (ADL)	Severe unsteadiness or sensation of movement; limiting self-care ADL; medical intervention required
Headache	Mild pain; No interference with activity.	Moderate pain; repeated use of non-narcotic pain reliever for more than 24 h OR some interference with activity.	Significant pain; any use of narcotic pain reliever OR prevents daily activity OR requires triptans.

Table 2 Toxicity Grading Tables – CLINICAL AEs

Clinical AEs	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Hypersomnia	Mild increased need for sleep	Moderate increased need for sleep	Severe increased need for sleep including excessive daytime sleepiness
Seizure	Brief partial seizure and no loss of consciousness	Brief generalized seizure	New onset seizures (partial or generalized); multiple seizures despite medical intervention
Somnolence	Mild but more than usual drowsiness or sleepiness	Moderate sedation; limiting instrumental ADL	Obtundation or stupor
Syncope	-	-	Fainting; orthostatic collapse
Tremor	Mild symptoms	Moderate symptoms; limiting instrumental ADL	Severe symptoms; limiting self-care
Other	Asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated	Moderate; minimal, local or noninvasive intervention indicated, limiting age-appropriate instrumental ADL	Severe or medically significant but not immediately life threatening; hospitalization or prolongation of existing hospitalization indicated; limiting self-care
PSYCHIATRIC DISORDERS			
Altered Mental Status: Includes agitation, confusion, disorientation, decreased mental acuity, changed mental status, somnolence, stupor			
Agitation	Mild mood alteration	Moderate mood alteration	Severe agitation; hospitalization not indicated
Confusion	Mild disorientation	Moderate disorientation; limiting instrumental ADL	Severe disorientation; limiting self-care
Insomnia	Mild difficulty falling asleep, staying asleep or waking up early	Moderate difficulty falling asleep, staying asleep or waking up early	Severe difficulty in falling asleep, staying asleep or waking up early; medical intervention required
Psychiatric disorders - Other, specify	Asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated	Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL	Severe or medically significant but not immediately life-threatening; limiting self-care; psychiatric intervention required
LOCAL IV CATHETER REACTION			
IV site reaction	Not applicable.	Erythema with associated symptoms (e.g., edema, pain, induration, phlebitis).	Ulceration or necrosis; severe tissue damage; operative intervention indicated.

Table 2 Toxicity Grading Tables – CLINICAL AEs

Clinical AEs	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
SYSTEMIC REACTIONS			
Anaphylaxis **	--	--	Symptomatic bronchospasm, with or without urticaria; parenteral intervention indicated; allergy-related edema or angioedema; hypotension.
***Definition*: A disorder characterized by an acute inflammatory reaction resulting from the release of histamine and histamine-like substances from mast cells, causing a hypersensitivity immune response. Clinically, it presents with breathing difficulty, dizziness, hypotension, cyanosis, and loss of consciousness, and may lead to death.*			
Allergic Reaction	Pruritus without rash.	Localized urticaria OR requires oral therapy.	Generalized urticaria OR angioedema OR anaphylaxis OR requires epinephrine
Hypersensitivity (including drug fever)	Transient flushing or rash; temperature 38.0 – 38.4°C (100.4 – 101.1°F).	Rash; flushing; urticaria; dyspnea; temperature 38.5 – 38.9°C (101.2 – 102.0°F).	Symptomatic bronchospasm with or without urticaria; parenteral medication indicated; allergy-related edema or angioedema; hypotension; temperature >38.9°C (>102.0°F).
Fatigue	No interference with activity.	Some interference with activity.	Significant; prevents daily activity.
Myalgia	No interference with activity.	Some interference with activity.	Significant; prevents daily activity.
SKIN			
Mucocutaneous	Erythema, pruritus.	Diffuse, maculo-papular rash, dry desquamation.	Vesiculation OR moist desquamation OR ulceration.
Pruritus	No or minimal interference with usual social and functional activities.	Greater than minimal interference with usual social and functional activities.	Inability to perform usual social and functional daily activities.
ALL OTHER CONDITIONS			
Illness or clinical AE (as defined according to applicable regulations)	Require minimal or no treatment; does not interfere with the subject's daily activities.	Result in a low level of inconvenience or concern with therapeutic measures; may cause some interference with functioning.	Interrupt a subject's usual daily activity and may require systemic drug therapy or other treatment; are usually incapacitating.

Table 3 Toxicity Grading Tables – LABORATORY AEs

Laboratory AEs	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Blood, serum, or plasma *			
HEMATOLOGY			
Hemoglobin decrease, Female, 18Y – g/dL	9.5 – 11.4	7.9 – 9.4	<7.9
Hemoglobin decrease, Female, >18Y – g/dL	9.7 – 11.6	8.1 – 9.6	<8.1
Hemoglobin decrease, Male, 18Y – g/dL	10.0 – 11.9	8.0 – 9.9	<8.0
Hemoglobin decrease, Male, >18Y – g/dL	11.2 – 13.1	9.2 – 11.1	<9.2
WBC increase 18Y – $x10^3$/ µL	13.1 – 15.0	15.1 – 20.0	>20.0
WBC increase >18Y – $x10^3$/ µL	10.9 – 15.0	15.1 – 20.0	>20.0
WBC decrease 18Y – $x10^3$/ µL	3.0 – 4.4	1.5 – 2.9	<1.5
WBC decrease >18Y – $x10^3$/ µL	2.3 – 3.7	1.1 – 2.2	<1.1
Neutrophils decrease 18Y – cells/ µL	1,200 – 1,799	750 – 1,199	<750
Neutrophils decrease >18Y – cells/ µL	1,000 – 1,499	750 – 999	<750
Lymphocytes decrease 18Y– cells/ µL	850 – 1,199	400 – 849	<400
Lymphocytes decrease >18Y – cells/ µL	500 - 849	300 - 499	<300
Monocytes increase – 18Y – cells/ µL	901 – 2,000	2,001 – 3,000	>3,000
Monocytes increase >18Y – cells/ µL	951 – 2,000	2,001 – 3,000	>3,000
Eosinophils increase >6Y– cells/ µL	501 – 750	751 – 1,000	>1,000
Basophils increase >6Y– cells/ µL	201 – 500	501 – 800	>800
Platelets decrease – $x10^3$/ µL	90 – <140	55 – <90	<55
COAGULATION			
PT INR	>1.1 – 1.8	>1.8 – 2.1	>2.1
Prothrombin Time - sec	>11.5 – 15.0	>15.0 – 18.6	>18.6
Activated Partial Thromboplastin Time (APTT) - sec	>32 – 54	>54 – 75	>75
CHEMISTRY			
Sodium decrease – mmol/L	130 - 134	124 –129	<124
Sodium increase – mmol/L	147 - 150	151 – 156	>156
Potassium increase, 19Y - mmol;/L	5.2 – 6.0	6.1 – 6.5	>6.5
Potassium increase,	5.4 – 6.0	6.1 – 6.5	>6.5

Table 3 Toxicity Grading Tables – LABORATORY AEs

Laboratory AEs	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
>19Y - mmol/L			
Potassium decrease, 19Y – mmol/L	3.2 – 3.7	2.6 – 3.1	<2.6
Potassium decrease, >19Y - mmol/L	3.0 – 3.4	2.5 – 2.9	<2.5
Carbon dioxide increase – mmol/L	33 – 36	37 – 40	>40
Carbon dioxide decrease – mmol/L	17 – 19	14 – 16	<14
Calcium increase, Male, 4 -19Y – mg/dL	10.5 – 11.2	11.3 – 12.3	>12.3
Calcium increase, Male, 20 - 49Y – mg/dL	10.4 – 11.4	11.5 – 12.5	>12.5
Calcium increase, Female, 4 -19Y – mg/dL	10.5 – 11.4	11.5 – 12.5	>12.5
Calcium increase, Female 20 - 49Y – mg/dL	10.3 – 11.4	11.5 – 12.5	>12.5
Calcium decrease, Male, 4 -19Y – mg/dL	8.1 – 8.8	7.3 – 8.0	<7.3
Calcium decrease, Male, 20 - 49Y – mg/dL	8.1 – 8.5	7.3 – 8.0	<7.3
Calcium decrease, Female, 4 -19Y – mg/dL	8.1 – 8.8	7.3 – 8.0	<7.3
Calcium decrease, Female, 20-49Y – mg/dL	8.1 – 8.5	7.3 – 8.0	<7.3
Blood urea nitrogen (BUN) increase, 19Y - mg/dL	21 – 58	59 – 120	>120
Blood urea nitrogen (BUN) increase, >19Y- mg/dL	26 – 58	59 – 120	>120
Glucose decrease, fasting – mg/dL	47 – 64	40 – 46	<40
Glucose increase, fasting – mg/dL	100 – 160	161 – 250	>250
Glucose increase, non-fasting – mg/dL	140 – 200	201 – 250	>250
Creatinine increase, Male, 18 - 29Y – mg/dL	1.25 – 1.62	1.63 – 2.24	>2.24
Creatinine increase, Male, 30 – 39 Y – mg/dL	1.27 – 1.64	1.65 – 2.27	>2.27
Creatinine increase, Male, 40 - 49Y – mg/dL	1.30 – 1.68	1.69 – 2.32	>2.32
Creatinine increase, Female, 18 - 29Y – mg/dL	0.97 – 1.25	1.26 – 1.73	>1.73
Creatinine increase, Female, 30 - 39Y – mg/dL	0.98 – 1.26	1.27 – 1.75	>1.75
Creatinine increase, Female, 40 - 49Y – mg/dL	1.00 – 1.29	1.30 – 1.78	>1.78
Direct bilirubin	0.3 – 0.6	0.7 – 1.2	>1.2
Total bilirubin (serum) increase (with other LFTs in the normal range), 19Y – mg/dL	1.2 – 1.7	1.8 – 2.5	>2.5
Total bilirubin (serum) increase	1.3 – 1.8	1.9 – 2.6	>2.6

Table 3 Toxicity Grading Tables – LABORATORY AEs

Laboratory AEs	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
(with other LFTs in the normal range), >19Y – mg/dL			
Total bilirubin (serum) increase (accompanied by a >3 x ULN increase in ALT or AST), 19Y – mg/dL	1.2 – 2.0	2.1 – 2.8	>2.8
Total bilirubin (serum) increase (accompanied by a >3 x ULN increase in ALT or AST), >19Y – mg/dL	1.3 – 2.1	2.2 – 3.0	>3.0
Total protein decrease, 19Y – g/dL	5.3 – 6.2	4.5 – 5.2	<4.5
Total protein decrease, >19Y – g/dL	5.1 – 6.0	4.3 – 5.0	<4.3
Albumin, decrease - g/dL	3.0 – 3.5	2.0– 2.9	<2.0
AST increase, 19Y – U/L	33 – 64	65 – 96	>96
AST increase, 20-49Y – U/L	41 – 80	81 – 120	>120
ALT increase, Male, 19Y – U/L	47 – 92	93 – 138	>138
ALT increase, Male, >19Y – U/L	47 – 92	93 – 138	>138
ALT increase, Female, 19Y – U/L	33 – 64	65 – 96	>96
ALT increase, Female, >19Y – U/L	30 – 58	59 – 87	>87
Alkaline phosphatase (AP) increase, Male, 19Y – U/L	170 – 338	339 – 507	>507
Alkaline phosphatase (AP) increase, Male, ≤49Y – U/L	131 – 260	261 – 345	>345
Alkaline phosphatase (AP) increase, Female, 19Y – U/L	129 – 256	257 – 384	>384
Alkaline phosphatase (AP) increase, Female, ≤49Y – U/L	126 – 250	251 – 375	>375
Urine			
URINALYSIS by Dipstick			
Protein	1+	2+	>2+
Blood (occult)	1+	2+	>2+
Glucose	1+	2+	>2+
Leukocyte esterase	1+	2+	>2+
URINE MICROSCOPY			
Red blood cells (RBC) per HPF	3 – 10	11 – 40	>40 and/or gross blood
WBC (microscopic) – WBC per HPF	6 – 10	11 – 40	>40 and/or symptomatic urogenital infection
Bacteria (microscopic)	few	moderate	many

Note 1: With the exception of AST, ALT, AP, total and direct bilirubin, BUN, creatinine, CL_{CR} (by the Cockcroft-Gault method), and urine protein, which should be within reference range, lab values of other analytes in the grade 1 range are acceptable for enrollment if (a) they are not considered to be clinically significant by the investigator and (b) there is no cluster of abnormal labs that combined are suggestive of an underlying disorder.

Note 2: Other Exceptions to screening laboratory tests' normal reference ranges are:

a. *Racially based low total WBC or neutrophil counts up to toxicity Grade 1 are allowed, but toxicity Grades 2 or 3 are exclusionary.*
b. *Labs performed as part of a panel but not listed above are to be recorded in the database. If abnormal, they are not exclusionary and are not to be graded per Toxicity table, however, the investigator would make a clinical decision about their clinical significance and, if clinically significant, they will be graded according to the criteria in Section 9.2.1. (Examples include mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), mean platelet volume (MPV), and nucleated red blood cell count (NRBC CT), which are included in a CBC with differential.)*

Note 3: If a subject was accepted into the trial with a laboratory value of an analyte that overlaps with values used for grading Grade 1 laboratory abnormalities, a TEAE will be reported if the on-study value of the same analyte increases in severity to Grade 2 or higher compared to the baseline.

Note 4: If the dipstick UA is abnormal for blood, protein, glucose and leukocyte esterase, a microscopic UA will be performed, and the results will supersede the results of the dipstick UA.

Note 5: Menstruating females with a positive dipstick UA or microscopic UA may be retested following cessation of menses. Gross blood in urine that is confirmed due to menses is not a TEAE (but is for all other reasons.

Note 6: Isolated *laboratory values that are outside the range of eligibility but are thought to be due to an acute condition or due to collection or laboratory error may be repeated once.*

Note 7: For the microscopic UA, Lab-reported ranges 3 -10 for RBC and 6-10 for WBC would be Grade 1; 10-20 and 20-40 for both RBC and WBC would be Grade 2; and > 40 for both RBC and WBC would be Grade 3.

Table 4 Toxicity Grading Tables – ECG

ECG interval abnormality	Reference range	Grade 1	Grade 2	Grade 3
QTcF interval prolonged (msec): • Male • Female	 • ≤450 msec • ≤470 msec	Asymptomatic, QTcF • 451 - 479 msec • 471 - 479 msec	Asymptomatic, QTcF 480-500 msec OR increase in interval 30-59 msec above baseline	Asymptomatic, QTcF >500 msec OR increase in interval ≥60 msec above baseline
PR interval prolonged (msec)	≤ 210 msec	211-250 msec	>250 msec	Type II 2nd degree AV block OR ventricular pause >3.0 sec

Note 1: Events will be coded as treatment-emergent SAEs if there are life-threatening associated symptoms or signs (arrhythmia, CHF, hypotension, syncope, TdP, etc.).

Note 2: If a male subject was accepted into the trial with a QTcF value that overlaps with values used for grading Grade 1 QTcF prolongation, a TEAE will be reported if the QTcF value is higher than the baseline value.

APPENDIX C: BLOOD VOLUME WITHDRAWN DURING THE TRIAL

Table 5: Laboratory Samples and Estimated Total Blood Volume (mL)

Study Periods	Out-patient	In-patient	In-patient Dosing Period (Days 1 – 7)								Out-patient	
Study Visit	Screen	Check-in	Dosing							Dis-charge	Follow-up	ET
Study Day [a]	-28 to -2	-1	1	2	3	4	5	6	7	8	11 (+3 days)	
HEMATOLOGY [1]	4	4		4		4		4		4	4	4
COAGULATION [1]	2.7	2.7		2.7		2.7		2.7		2.7	2.7	2.7
CHEMISTRY [1]	8.5	8.5		8.5		8.5		8.5		8.5	8.5	8.5
Serum β-HCG and FSH [1]	0											
Viral Serology (HIV, HBsAg, HCV) [3]	8.5											
PK [4]			100	20	20	20	20	20	100	10		10
Total volume/visit	23.7	15.2	100	35.2	20	35.2	20	35.2	100	25.2	15.2	31.2
Cumulative total volume	23.7	38.9	138.9	174.1	194.1	229.3	249.3	284.5	384.5	409.7	424.9	

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

[1] Clinical Safety blood tests (HEM, CHEM, COAG) are drawn at Screening Visit, on Day -1, pre-dose on Days 2, 4, and 6, on Days 8 and 11 (+ 3 days), or ET. The test includes serum β-HCG and FSH.

[2] Serum pregnancy test (β-HCG) in all women at Screening. FSH at Screening only in post-menopausal women.

[3] Viral serology tests are drawn at Screening.

[4] PK plasma samples are drawn on:
Day 1 and Day 7: Before and at 7- 9 other timepoints after the start of infusion, depending on duration of infusion (See Appendix A and Section 8.3.1); Day 2 to Day 8: (daily, 24 h after starting the infusion on the previous day, Day 1 to Day 7); Day 2 to Day 6 (daily, 12 h after the dose), or ET if occurs within 24 h of dosing.