A Phase I, Open-Label Study in Healthy Adults to Evaluate the Safety and Pharmacokinetics of AVYCAZ® in Combination with Aztreonam (COMBINE)

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STATEMENT OF ASSURANCE

Each Institution will hold a current Federal Wide Assurance (FWA) issued by the Office of Human Research Protections (OHRP) for federally-funded human subject's research. Each FWA will designate at least one Institutional Review Board (IRB)/Independent Ethics Committee (IEC) registered with OHRP, for which the research will be reviewed and approved by the IRB/IEC and will be subject to continuing review [45 CFR 46.103(b)]. The IRB/IEC designated under an FWA may include an institution's IRB/IEC, an independent IRB/IEC, or an IRB/IEC of another institution after establishing a written agreement with that other institution.

STATEMENT OF COMPLIANCE

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

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

SIGNATURE PAGE

The signature below provides the necessary assurance that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable US federal regulations and ICH E6 Good Clinical Practice (GCP) guidelines.

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

I agree to conduct the study in accordance with the current protocol and will not make changes to the protocol without obtaining the sponsor's approval and IRB/IEC approval, except when necessary to protect the safety, rights, or welfare of subjects.

Site Investigator Signature:*

Signed: _____ Date: _____

Jeff Guptill, M.D., MA, MHS

Associate Professor, Duke Clinical Research Institute (DCRI)

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LIST OF ABBREVIATIONS

α	Alpha
AE	Adverse Event/Adverse Experience
ALP	Alkaline Phosphatase
ALT	Alanine Transaminase
AM	Ante Meridiem
AST	Aspartate Aminotransferase
ARLG	Antibacterial Resistance Leadership Group
ATM	Aztreonam
AUC	Area Under the Curve
$AUC_{(0\text{-}Tau)}$	Area under the plasma concentration-time curve during the dosing interval on Day 1
$AUC_{0\text{-}Tau,ss}$	Area under the plasma concentration-time curve during the dosing interval at steady state
AV	Atrioventricular
AVI	Avibactam
BMI	Body Mass Index
BPM	Beats Per Minute
BUN	Blood Urea Nitrogen
°C	Degrees Celsius
C_{max}	Maximum Plasma Concentration
$C_{ss,max}$	Maximum Plasma Concentration at Steady State
$C_{ss,max(tss,max)}$	Time to Maximum Plasma Concentration at Steady State
$C_{ss,min}$	Minimum Plasma Concentration at Steady State
$C(t_{max})$	Time to Maximum Plasma Concentration

CAZ	Ceftazidime
CFR	Code of Federal Regulations
CFU	Colony Forming Unit
CI	Continuous Infusion
cIAI	Complicated Intraabdominal Infection
Cl	Chloride
CL	Systemic Plasma Clearance
cm	Centimeters
CRF	Case Report Form
CROMS	Clinical Research Operations and Management Support
CS	Clinically Significant
cUTI	Complicated Urinary Tract Infection
DCRI	Duke Clinical Research Institute
DEPRU	Duke Early Phase Research Unit
DHHS	Department of Health and Human Services
dL	Deciliter
DMID	Division of Microbiology and Infectious Diseases, NIAID, NIH, DHHS
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
ESBL	Extended-spectrum β-lactamases
°F	Degrees Fahrenheit
FDA	Food and Drug Administration
FEV1	Forced Expiratory Volume in 1 Second
FWA	Federal Wide Assurance
g	Gram

GCP	Good Clinical Practice
GNB	Gram Negative Bacteria
HBsAg	Hepatitis B Surface Antigen
HCO_3	Bicarbonate
HCV	Hepatitis C Virus
hCG	Human Chorionic Gonadotropin
HEENT	Head, Eye, Ear, Nose, Throat
HFIM	Hollow Fiber Infection Model
HLT	High Level Term
HIV	Human Immunodeficiency Virus
hpf	High Power Field
IATA	International Air Transport Association
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IDS	Investigational Drug Services
IEC	Independent or Institutional Ethics Committee
IND	Investigational New Drug Application
IRB	Institutional Review Board
ISM	Independent Safety Monitor
IV	Intravenous
K/CUMM	Liters/mm^3
K	Potassium
kg	Kilogram
KPC	*Klebsiella pneumoniae* Carbapenemase
L	Liters

LCE	Leukocyte Esterase
LDH	Lactate Dehydrogenase
LFT	Liver Function Test
LLC	Limited Liability Company
m^2	Meters Squared
MBL	Metallo-β-Lactamase
MD	Maryland
M.D.	Medical Doctor
MedDRA®	Medical Dictionary for Regulatory Activities
mg	Milligram
mEq	Milliequivalent
mL	Milliliter
MM	Medical Monitor
mmHg	Millimeter of Mercury
MOP	Manual of Procedures
msec	Millisecond
Na	Sodium
NC	North Carolina
NCA	Non-compartmental Analysis
NCS	Not Clinically Significant
NIAID	National Institute of Allergy and Infectious Diseases, NIH, DHHS
NIH	National Institutes of Health
OER	Office of Extramural Research
OHRP	Office of Human Research Protection

PD	Pharmacodynamic
pH	Potential of Hydrogen
PI	Principal Investigator
PK	Pharmacokinetics
PMC	Pharmacometrics Center
PR	Period on ECG extending from beginning of the P wave (the onset of atrial depolarization) until the beginning of the QRS complex
PT	Prothrombin Time
PTT	Partial Thromboplastin Time
PubMed	Public/Publisher MEDLINE
PVC	Premature Ventricular Contraction
QA	Quality Assurance
QC	Quality Control
QT	Period on ECG extending from the beginning of the QRS complex to the end of the T wave
QTc	QT interval, corrected for Heart Rate
QTcF	QT duration corrected for heart rate by Fridericia's formula
$RAUC_{(0\text{-}Tau)}$	Accumulation ratio for $AUC_{(0\text{-}Tau)}$
RBC	Red Blood Cell
RC_{max}	Accumulation ratio for C_{max}
SAE	Serious Adverse Event/Serious Adverse Experience
SDCC	Statistical and Data Coordinating Center
SMC	Safety Monitoring Committee
t_{max}	Time for Maximum Concentration of Drug
U	Units

ULN	Upper Limit of Normal
USA	United States of America
US	United States
USP	United States Pharmacopeia
V_{ss}	Volume of Distribution at Steady-State
V_z	Volume of Distribution at the Terminal Phase
WBC	White Blood Cell
w/v	Weight/Volume

PROTOCOL SUMMARY

Title:	A Phase I, Open-Label Study in Healthy Adults to Evaluate the Safety and Pharmacokinetics of AVYCAZ® in Combination with Aztreonam (COMBINE)
Design of the Study:	This is a Phase I, open-label, single center study in 48 healthy adult male and female subjects to investigate the safety and pharmacokinetics of ceftazidime-avibactam (AVYCAZ) combined with aztreonam (ATM), AVYCAZ alone, and ATM alone. Cohorts 1-4 are the single drug administration treatment cohorts and will include AVYCAZ per label dosing, AVYCAZ as a continuous infusion (CI), ATM per label dosing, and ATM as a CI. Cohorts 5 and 6 are the two AVYCAZ and ATM combination drug administration treatment cohorts. Study safety will be monitored using assessments of adverse events (AE's), vital signs, and clinical laboratory safety tests. Serial blood and urine samples will be collected for pharmacokinetic (PK) evaluation.
Study Phase:	1
Study Population:	Forty-eight (48) healthy, adult male and female subjects ages 18-45. The study will be conducted in the United States.
Number of Sites:	1
Description of Study Product or Intervention:	Eight (8) subjects/cohort. • Cohort 1: AVYCAZ 2.5 g IV as a 2-hour infusion every 8 hours for 7 days • Cohort 2: AVYCAZ 2.5 g IV as a 2-hour infusion x 1, then 0.32 g per hour IV daily as a CI (7.5 g/day) for 7 days • Cohort 3: ATM 2 g IV as a 2-hour infusion every 6 hours for 7 days • Cohort 4: ATM 2 g IV as a 2-hour infusion x 1, then 0.33 g per hour IV daily as a CI (8 g/day) for 7 days

- Cohort 5: AVYCAZ 2.5 g IV as a 2-hour infusion every 8 hours for 7 days and ATM 1.5 g IV as a 2-hour infusion every 6 hours for 7 days
- Cohort 6: AVYCAZ 2.5 g IV as a 2-hour infusion every 8 hours for 7 days and ATM 2 g as a 2-hour infusion every 6 hours for 7 days

Study Objectives:

Primary:

- Describe the safety of two dosing regimens of AVYCAZ combined with ATM relative to AVYCAZ alone and ATM alone in healthy adult subjects.

Secondary:

- Characterize the PK profiles of two dosing regimens of AVYCAZ combined with ATM, AVYCAZ alone, and ATM alone at the population level in healthy adult subjects.
- Characterize the PK profiles of two dosing regimens of AVYCAZ combined with ATM, AVYCAZ alone, and ATM alone following initiation of dosing on Day 1 in healthy adult subjects.
- Characterize the PK profiles of two dosing regimens of AVYCAZ combined with ATM, AVYCAZ alone, and ATM alone following multiple daily dosing in healthy adult subjects.

Exploratory:

- Predict the distribution of plasma concentration-time profiles observed with two dosing regimens AVYCAZ combined with ATM, AVYCAZ alone, and ATM alone in healthy adult subjects via simulations.
- Predict the distribution of cumulative urine amount-time profiles observed with two dosing regimens AVYCAZ combined with ATM, AVYCAZ alone, and ATM alone in healthy adult subjects via simulations.
- Examine the associations between the plasma concentration-time profiles of AVYCAZ combined with ATM, AVYCAZ

	alone, and ATM alone and occurrence of ALT or AST elevations.
Duration of Individual Subject Participation:	Up to 44 days (Screening: within 30 days of Day 1 of study product administration; study product administration: 7 days; outpatient follow-up visit: Day 11 +3 days post Day 1 of study product administration).
Estimated Time to Last Subject/Last Study Day:	The study will run between approximately 12-15 months.

1 KEY ROLES

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2 BACKGROUND AND SCIENTIFIC RATIONALE

2.1 Background

Treatment of patients with serious infections due to highly resistant Gram-negative bacteria (GNB) remains problematic and is a major public health concern. Not only is there increased resistance among frequently encountered GNB like *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*, but there is also a rise in the number of multidrug-resistant strains [1-3]. This change is driven in large part by the increasing prevalence and complexity of a variety of β-lactamases [4]. The most important of these are the extended-spectrum β-lactamases (ESBLs) and carbapenemases, such as *Klebsiella pneumoniae* carbapenemase (KPC) [1,3,4]. The metallo-β-lactamases (MBLs), which have the ability to hydrolyze all β-lactams except aztreonam (ATM), have emerged recently as problematic carbapenemases [5-10]. While there has been an impressive drug development response to combat ESBL- and KPC-producing GNB infections, none of the recently approved antibiotics have reliable activity against MBL-producing GNB [11,12]. Several antibiotics with activity against MBL-producing GNB are being developed, but none are anticipated to be available until at least 2021 [11,13].

Previous studies have demonstrated that while ATM is not hydrolyzed by MBLs, many MBL-bearing GNB co-harbor ESBLs that hydrolyze ATM [6,7,9,13,15]. Combination with avibactam (AVI), a beta-lactamase inhibitor, protects ATM from hydrolysis by ESBLs and KPCs and results in effective killing of bacteria harboring a broad range of beta-lactamase enzymes [5-8,10,13,15]. The ATM-AVI combination is currently being tested in clinical trials; however, it will be several years before this new treatment option will be considered for approval by the Food and Drug Administration (FDA) for treating patients infected with MBL-producing GNB. This underscores the exigency of redeploying our existing agents in innovative ways to meet the needs of patients today [14].

One strategy that may serve as a "bridge" treatment for MBL-producing GNB infections is ceftazidime-avibactam (AVYCAZ®) combined with ATM [5,13,16,17]. In the combination of ATM with AVYCAZ, AVI inhibits the ESBL and KPC beta-lactamases that are often present in MBL-producing GNB, allowing ATM, which is unaffected by MBLs, to effectively bind to bacterial penicillin binding proteins. Limited data to date suggest that AVYCAZ combined with ATM is a promising treatment for patients with MBL-producing GNB infections [5,10,13,17]. In a MBL-producing *K. pneumoniae* time-kill assay, AVYCAZ combined with ATM resulted in a ≥ 3-log10-colony forming unit (CFU)/mL decrease compared to AVYCAZ alone [13]. In a murine neutropenic thigh infection model with the same strain, an almost 4-log10-CFU/mL greater reduction was noted with AVYCAZ combined with ATM vs. AVYCAZ alone [13]. Although the precise mechanism of improved bacterial killing activity with AVYCAZ combined with ATM is not completely understood, it is likely attributable to maximal saturation of the

diverse penicillin binding proteins present in GNB, flooding of periplasm with β-lactams, and maximal binding of available β-lactamases [5,14].

The combination of AVYCAZ with ATM has been employed clinically. To date, four published case reports have documented successful use of this combination in 14 patients with serious infections due to MBL-producing GNB [5,13,16,17]. Patients have received this combination for infections due to MBL-bearing strains of *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Pseudomonas aeruginosa*, and *Stenotrophomonas maltophilia*. While there are theoretical safety concerns with dual beta-lactam use, no adverse events (AEs) were attributed to receipt of AVYCAZ combined with ATM across the published patient reports [5,13,14, 16,17]. This is notable as at least two of the patients described in these case reports received AVYCAZ combined with ATM for greater than one month [13,17].

Although AVYCAZ combined with ATM has been shown to be efficacious against MBL-producing GNB in pre-clinical studies and clinical case reports, optimal dosing remains unknown. Hollow fiber infection model (HFIM) studies using *E. coli* ARLG-1013 ($bla_{\text{NDM-1}}$, $bla_{\text{CTX-M}}$, bla_{CMY}, bla_{TEM}) and *K. pneumoniae* ARLG-1002 ($bla_{\text{NDM-1}}$, $bla_{\text{CTXM-15}}$, bla_{DHA}, bla_{SHV}, bla_{TEM} were conducted to identify AVYCAZ combined with ATM regimens that result in maximal bacterial kill and resistance suppression [23]. In these HFIM experiments, a number of FDA-approved ATM 6-8 grams (g)/day regimens were evaluated in combination with FDA-approved AVYCAZ dosing (2.5 g IV as a 2-hour infusion every 8 hours) and continuous infusion (CI) AVYCAZ (7.5 g IV daily) [18,19]. It was anticipated that administration of AVYCAZ as a CI combined with ATM would outperform FDA-approved AVYCAZ dosing combined with ATM. Continuous infusion administration maximizes the pharmacokinetics/ pharmacodynamics (PK/PD) of AVYCAZ and it was anticipated that this would result in enhanced bacterial killing and resistance suppression [20-22]. Since it is not practical for all institutions to infuse AVYCAZ as a CI, HFIM experiments were also conducted to identify the optimal dosing of AVYCAZ combined with ATM using FDA-approved dosing regimens of each antibiotic. In these HFIM experiments, maximal daily dose of AVYCAZ combined with 8g/day ATM regimens were found to be optimal. Specifically, the two combination regimens that showed maximal bacterial killing and resistance suppression over 7 days were:

- AVYCAZ 2.5 g IV as a 2-hour infusion every 8 hours combined with ATM 2 g IV as a 2-hour infusion every six hours, and
- AVYCAZ combined with ATM, each administered as a CI (AVYCAZ 7.5 g/day CI combined with ATM 8g/day CI) [23].

While the initial intent was to examine the two optimal AVYCAZ with ATM regimens identified in the HFIM experiments, two subjects who received ATM 8g/day CI alone in cohort 4 experienced grade 3 AST/ALT elevations. Despite having AST/ALT elevations >10 ULN, the two subjects were asymptomatic and there were no clinical findings suggestive of liver necrosis

or jaundice. There were also no elevations in ALP and bilirubin and there were no clinically significant elevations in all other serum chemistries and blood counts. The two subjects' in cohort 4 continued to experience elevations in AST/ALT for 1 to 2 days after discontinuation of ATM but the AST/ALT values then trended down 2 to 4 days after study product discontinuation. For both subjects, AST/ALT values returned to baseline within 25 days after discontinuation of ATM. Please note that for both subjects, daily AST/ALT monitoring was not performed as subjects had been discharged from the study unit and only returned for two additional AE follow up visits 5-9 and 25 days after discontinuation of ATM.

Asymptomatic serum aminotransferase elevations are common during high dose, intravenous ATM therapy (10% to 38%) [24]. Liver enzyme elevations occur slightly more commonly during aztreonam therapy than with other comparative antibiotics. As observed in the two subjects in cohort 4 with grade 3 AST/ALT elevations, aztreonam induced liver injury appears to be transient, mild and asymptomatic, marked only by serum enzyme elevations. Data indicate that aztreonam is an unlikely cause of clinically apparent liver injury (likelihood score of E) and no individual case of frank liver injury and jaundice attributable to aztreonam have been reported.

In response to the occurrence of two grade 3 AEs that were related to study product and of the same type [High Level Term (HLT)], the study was halted and a Safety Monitoring Committee (SMC) meeting was convened. The SMC recommended to continue the study but reduce the dose of ATM in cohort 5 to ATM 1.5 g IV as a 2-hour infusion every 6 hours (ATM 6 g/daily) and to administer it in combination with AVYCAZ 2.5 g IV as a 2-hour infusion every 8 hours. The HFIM study indicated that this was an effective combination regimen and SMC recommended administering it in cohort 5. Furthermore, the SMC recommended that a SMC meeting be convened after completion of cohort 5. If no halting rules are met and there are no other safety concerns, the SMC recommended administering ATM 2 g IV as a 2-hour infusion every 6 hours (ATM 8 g/daily) in combination with AVYCAZ 2.5 g IV as a 2-hour infusion every 8 hours in cohort 6. The SMC was in favor of escalating the ATM dose in cohort 6 if no halting rules are met as the HFIM experiments showed increased bacterial killing with 8 g/day vs 6 g/day combination regimens. Since the safety of ATM CI was not established in this study, the SMC was opposed to administering ATM as a CI in any subsequent cohorts. As an additional safeguard, the SMC also recommended that more intensive Liver Function Test (LFT) monitoring be performed.

2.2 Scientific Rationale

2.2.1 Purpose of Study

Before uniform adoption of AVYCAZ with ATM combination regimens that were found to be effective in the aforementioned HFIM studies into clinical practice, it is important to establish

the safety and PK of these regimens in humans [23]. This is critically important as two subjects in the ATM 8g/daily CI treatment cohort (cohort 4) experienced asymptomatic grade 3 AST/ALT elevations. Therefore, this phase 1 study in healthy adults will evaluate the safety and PK of AVYCAZ combined with ATM. AVYCAZ and ATM are both currently approved as standalone products for the treatment of patients with various infections due to GNB. Both antibiotics are generally safe and well-tolerated, although mild-to-moderate asymptomatic serum aminotransferase elevations are common with ATM [18, 19, 24]. These elevations are usually self-limiting and do not require ATM discontinuation [18, 24]. Although AVYCAZ and ATM appear to be safe and well-tolerated, there are no available data on safety when these antibiotics are used in combination. It is unclear if AVYCAZ combined with ATM will further exacerbate liver enzyme elevations or lead to other AEs due to the potential of cumulative toxicity from dual-β-lactam treatment [14]. There are also no published PK data of these antibiotics when administered concurrently, and it is therefore unknown if use of these agents in combination will lead to an altered PK profile of each agent due to inhibition of renal or other compensatory clearance mechanisms. Therefore, it is important to assess the safety and pharmacokinetic profile of AVYCAZ combined with ATM relative to its standalone counterparts before widespread adoption into clinical practice.

2.2.2 Study Population

The study will be conducted using healthy volunteers to observe the safety and PK of combination regimens of AVYCAZ with ATM that were found to be effective in previous HFIM experiments [23]. Safety and PK of combination regimes will be compared to the AVYCAZ and ATM dosing regimens given alone [23]. This is a single center study being conducted in the United States. The study site will determine the most efficient procedures to identify potentially eligible subjects from the general public for the study. Women of child-bearing potential will not be excluded from the study. In addition, special populations, e.g., non-English speakers, illiterate or non-writing individuals will not be excluded from this study.

Children and vulnerable populations will be excluded from this study as there is insufficient safety data and this is the first time AVYCAZ combined with ATM will be administered in a systematic manner and as there is insufficient safety data to support the inclusion of these vulnerable populations.

2.3 Potential Risks and Benefits

AVYCAZ and ATM are both currently FDA-approved and the package inserts are the primary source of risk information for AVYCAZ and ATM [18,19].

2.3.1 Potential Risks

2.3.1.1 AVYCAZ

Hypersensitivity Reactions: Serious and occasionally fatal hypersensitivity (anaphylactic) reactions and serious skin reactions have been reported in patients receiving beta-lactam antibacterial drugs. Before therapy with AVYCAZ is instituted, careful inquiry about previous hypersensitivity reactions to other cephalosporins, penicillins, or carbapenems should be made. Exercise caution if this product is to be given to a penicillin or other beta-lactam-allergic patient because cross-sensitivity among beta-lactam antibacterial drugs has been established. Discontinue the drug if an allergic reaction to AVYCAZ occurs.

Clostridium difficile-Associated Diarrhea: *Clostridium difficile*-associated diarrhea has been reported for nearly all antibiotics, including AVYCAZ, and may range in severity from mild diarrhea to fatal colitis. The diarrhea may start during or after the AVYCAZ is administered. *C. difficile* occurs naturally in the colon. Treatment with antibiotic drugs alters the normal flora (microorganisms that live in the colon) and may permit overgrowth of *C. difficile*.

Central Nervous System Reactions: Seizures, non-convulsive status epilepticus, encephalopathy, coma, asterixis, neuromuscular excitability, and myoclonia have been reported in patients treated with ceftazidime, particularly in the setting of renal impairment.

Nervous System: headache, dizziness

Gastrointestinal: abdominal pain, nausea, vomiting, diarrhea

The following selected adverse reactions were reported in AVYCAZ treated patients at a rate of less than 1% in the Phase 3 clinical trials and are not described elsewhere in the labeling.

- Blood and lymphatic disorders - thrombocytopenia
- General disorders and administration site conditions - injection site phlebitis
- Infections and infestations - candidiasis
- Investigations - increased aspartate aminotransferase (AST), increased alanine aminotransferase (ALT), increased gamma-glutamyltransferase
- Metabolism and nutrition disorders - hypokalemia
- Nervous system disorders - dysgeusia
- Renal and urinary disorders - acute renal failure, renal impairment, nephrolithiasis
- Skin and subcutaneous tissue disorders - rash, rash maculo-papular, urticaria, pruritus
- Psychiatric disorders - anxiety

Additionally, adverse reactions reported with ceftazidime alone that were not reported in AVYCAZ treated patients in the Phase 3 clinical trials are listed below:

- Blood and lymphatic disorders - agranulocytosis, hemolytic anemia, leukopenia, lymphocytosis, neutropenia, thrombocytosis, eosinophilia
- General disorders and administration site conditions - infusion site inflammation, injection site hematoma, injection site thrombosis
- Hepatobiliary disorders - jaundice
- Investigations - increased blood lactate dehydrogenase, prolonged prothrombin time (PT)
- Nervous system disorders - paresthesia
- Renal and urinary disorders - tubulointerstitial nephritis
- Reproductive and breast disorders - vaginal inflammation
- Skin and subcutaneous tissue disorders - angioedema, erythema multiforme, Stevens-Johnson syndrome, toxic epidermal necrolysis

Laboratory Changes: Seroconversion from a negative to a positive direct Coombs test result at any time up to the last visit occurred in 31/240 (12.9%) of patients receiving AVYCAZ plus metronidazole with initial negative Coombs test and at least one follow-up test and in 7/235 (3.0%) of patients receiving meropenem in the Phase 3 cIAI trial.

Seroconversion from a negative to a positive direct Coombs test result at any time up to the last visit occurred in 7/216 (3.2%) of patients receiving AVYCAZ with initial negative Coombs test and at least one follow-up test and 2/214 (0.9%) of patients receiving doripenem in the Phase 3 cUTI trial. No adverse reactions representing hemolytic anemia were reported in any treatment group.

Ceftazidime is excreted in human milk in low concentrations. It is not known whether AVI is excreted into human milk, although AVI was shown to be excreted in the milk of rats. No information is available on the effects of CAZ and AVI on the breast-fed child or on milk production.

2.3.1.2 Aztreonam

Asymptomatic serum aminotransferase elevations have been reported with ATM therapy (10% to 38%) [24]. The enzyme abnormalities are usually mild-to-moderate, asymptomatic, self-limited and not requiring drug discontinuation. Instances of marked aminotransferase elevations within 3 to 5 days of starting ATM have been reported, but these cases were without jaundice and resolved over time once the drug was stopped. ATM is an unlikely cause of clinically

apparent liver injury and no individual cases of frank liver injury and jaundice attributable to ATM have been reported.

Local reactions such as phlebitis/thrombophlebitis following intravenous (IV) administration; and discomfort/swelling at the injection site following intramuscular administration occurred at rates of approximately 1.9% and 2.4% respectively.

Systemic reactions (considered to be related to therapy or of uncertain etiology) occurring at an incidence of 1% to 1.3% include diarrhea, nausea and/or vomiting, and rash. Reactions occurring at an incidence of less than 1% are listed within each body system in order of decreasing severity:

- Hypersensitivity - anaphylaxis, angioedema, bronchospasm
- Renal - Prolonged serum levels of ATM may occur in patients with transient or persistent renal insufficiency.
- Hematologic - pancytopenia, neutropenia, thrombocytopenia, anemia, eosinophilia, leukocytosis, thrombocytosis
- Gastrointestinal - abdominal cramps; rare cases of *Clostridium difficile*-associated diarrhea, including pseudomembranous colitis, or gastrointestinal bleeding have been reported.
- Onset of pseudomembranous colitis symptoms may occur during or after antibiotic treatment.
- Dermatologic - toxic epidermal necrolysis, purpura, erythema multiforme, exfoliative dermatitis, urticaria, petechiae, pruritus, diaphoresis
- Cardiovascular - hypotension, transient electrocardiogram (ECG) changes (ventricular bigeminy and premature ventricular contraction (PVC), flushing
- Respiratory - wheezing, dyspnea, chest pain
- Hepatobiliary - hepatitis, jaundice
- Nervous System - seizure, confusion, vertigo, paresthesia, insomnia, dizziness
- Musculoskeletal - muscular aches
- Special Senses - tinnitus, diplopia, mouth ulcer, altered taste, numb tongue, sneezing, nasal congestion, halitosis
- Other - vaginal candidiasis, vaginitis, breast tenderness
- Body as a Whole - weakness, headache, fever, malaise

Laboratory Changes: Adverse laboratory changes without regard to drug relationship that were reported during clinical trials were:

- Hepatic - elevations of AST, Serum Glutamic Oxaloacetic Transaminase, ALT, Serum Glutamic-Pyruvic Transaminase, and alkaline phosphatase (ALP); signs or symptoms of hepatobiliary dysfunction occurred in less than 1% of recipients
- Hematologic - increases in prothrombin and partial thromboplastin times, positive Coombs test.
- Renal - increases in serum creatinine.

In pregnant women, ATM crosses the placenta and enters the fetal circulation. ATM is excreted in human milk in concentrations that are less than 1% of concentrations determined in simultaneously obtained maternal serum.

2.3.1.3 AVYCAZ Combined with ATM

The risks associated with AVYCAZ combined with ATM are expected to be similar to those associated with AVYCAZ alone and ATM alone. However, there is a theoretical risk for increased adverse reactions with AVYCAZ combined with ATM relative to that observed with receipt of AVYCAZ alone and ATM alone due to the potential of cumulative or additive toxicity from receiving two beta-lactams simultaneously relative to receiving just one beta-lactam [14].

To date, published reports have described the use of AVYCAZ combined with ATM in 14 patients [5,13,16,17]. No adverse reactions were attributed to receipt of AVYCAZ combined with ATM in these reports. Four of the 14 patients (28.6%) were reported to have died but none of the deaths were considered related to the patient's infection or receipt of the AVYCAZ combined with ATM.

2.3.1.4 Risks of Drawing Blood and Intravenous Infusions

Risks associated with drawing blood from the subjects' arm or administering IV fluids include momentary discomfort and/or bruising. Infection, excess bleeding, clotting, or fainting are also possible, although unlikely.

2.3.2 Potential Benefits

There are no benefits to healthy subjects for their inclusion in this protocol. However, data from this study may be helpful in determining safe treatment doses in patients with serious antibiotic resistant GNB infections.

3 STUDY DESIGN, OBJECTIVES AND ENDPOINTS OR OUTCOME MEASURES

3.1 Study Design Description

This is a Phase I, open-label, single center study in 48 healthy adult male and female subjects age 18-45 years old (6 cohorts of 8 subjects) to investigate the safety and PK of AVYCAZ combined with ATM, AVYCAZ alone, and ATM alone.

Following subject screening, eligible subjects will be assigned into one of the dosing cohorts. Subjects will be admitted to the study site on Day -1 for evaluations to confirm they continue to meet eligibility criteria, after which they will be admitted to the study site for dosing and observation. Subjects will stay for a minimum of 7 nights and 8 days. Cohorts 1-4 will be completed prior to Cohorts 5 and 6.

Six dosing cohorts will be evaluated (see Section 4.3). Four treatment cohorts are single agent dosing cohorts and will include AVYCAZ per label dosing, AVYCAZ as a CI, ATM per label dosing, and ATM as a CI. Single drug treatment cohorts are being conducted to collect baseline safety and pharmacokinetic data. The remaining two cohorts are two AVYCAZ combined with ATM regimens that were found to be effective in the aforementioned HFIM studies (see Section 2.1). In the treatment cohorts in which the antibiotics are administered as a CI, an initial loading dose will be administered prior to starting the CI. Since the maximum concentration of antibiotic (C_{max}) is lower and the time for maximum concentration of drug (t_{max}) is delayed with CI, the initial loading dose is included to shorten the time it takes to achieve critical PK/PD associated with maximal response [20,25,26]. The use of initial loading doses is also congruent with clinical practice guideline recommendations to use PK/PD data to optimize dosing early in the course of therapy for patients with serious, life threatening infections [27, 28].

Study safety will be closely monitored using daily assessments of AE's, vital signs and clinical laboratory safety tests. Clinical laboratory safety tests will be collected on Day -1, Days 2, 4, 6 and Day 8 (prior to discharge from the study site). LFTs will be obtained daily beginning on Day 4 until a subject completes dosing. The Final Visit (Day 11 + 3) will be scheduled with each subject for a final safety evaluation and collection of clinical laboratory safety tests.

Plasma and urine concentrations of AVYCAZ and ATM will be quantified, when each antibiotic is given alone (AVYCAZ or ATM), and in combination (AVYCAZ combined with ATM) following the first dose and after multiple administrations.

The study will run between approximately 12-15 months.

3.2 Study Objectives

3.2.1 Primary

- Describe the safety of two dosing regimens of AVYCAZ combined with ATM relative to AVYCAZ alone, and ATM alone in healthy adult subjects.

3.2.2 Secondary

- Characterize the PK profiles of two dosing regimens of AVYCAZ combined with ATM, AVYCAZ alone, and ATM alone at the population level in healthy adult subjects.
- Characterize the PK profiles of two dosing regimens of AVYCAZ combined with ATM, AVYCAZ alone, and ATM alone following initiation of dosing on day 1 in healthy adult subjects.
- Characterize the PK profiles of two dosing regimens of AVYCAZ combined with ATM, AVYCAZ alone, and ATM alone following multiple daily dosing in healthy adult subjects.

3.2.3 Exploratory

- Predict the distribution of plasma concentration-time profiles observed with two dosing regimens AVYCAZ combined with ATM, AVYCAZ alone, and ATM alone in healthy adult subjects via simulations.
- Predict the distribution of urine cumulative urine amount-time profiles observed with two dosing regimens AVYCAZ combined with ATM, AVYCAZ alone, and ATM alone in healthy adult subjects via simulations.
- Examine the associations between the plasma concentration-time profiles of AVYCAZ combined with ATM, AVYCAZ alone, and ATM alone and occurrence of ALT or AST elevations.

3.3 Study Endpoints or Outcome Measures

3.3.1 Primary

- Number of subjects in each treatment cohort with at least one Grade 2 or higher treatment-emergent AE from first dose through follow-up period.

3.3.2 Secondary

- Incidence and severity of all treatment-emergent AEs in each treatment cohort from first dose through follow-up period.

- Population mean PK parameter estimates and the magnitude of the associated inter-individual variability for AVYCAZ and ATM, when given alone and in combination.
- Individual post-hoc PK parameter estimates and calculated exposure measures in plasma for AVYCAZ and ATM, when given alone and in combination following initial dosing on Day 1.
- Individual post-hoc PK parameters estimates and calculated exposure measures in urine for AVYCAZ and ATM, when given alone and in combination following initial dosing on Day 1.
- Individual post-hoc PK parameter estimates and calculated exposure measures in plasma for AVYCAZ and ATM, when given alone and in combination following multiple daily dosing.
- Individual post-hoc PK parameters estimates and calculated exposure measures in urine for AVYCAZ and ATM, when given alone and in combination following multiple daily dosing.

3.3.3 Exploratory

- Distribution of simulated plasma concentration-time profiles associated with each treatment cohort.
- Distribution of simulated urine concentration-time profiles associated with each treatment cohort.
- Occurrence of ALT or AST elevations ≥ 3 times upper limit of normal (ULN) from first dose of study product through follow-up period.
- Occurrence of ALT or AST elevations ≥ 5 times ULN from first dose of study product through follow-up period.
- Changes in ALT or AST from first dose of study product through follow-up period.

4 STUDY INTERVENTION/STUDY PRODUCT

4.1 Study Product Description

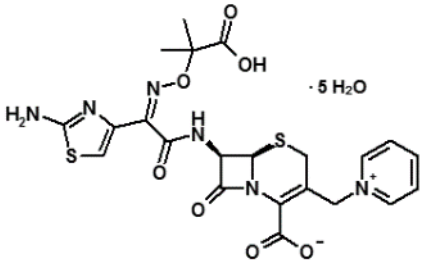
AVYCAZ:

AVYCAZ is an antibacterial combination product for intravenous administration consisting of the semisynthetic cephalosporin ceftazidime pentahydrate and the beta-lactamase inhibitor avibactam sodium at a fixed ratio of 4:1 [19].

- Ceftazidime

Ceftazidime is a semisynthetic, beta-lactam antibacterial drug. It is the pentahydrate of (6*R*,7*R,Z*)-7-(2-(2-aminothiazol-4-yl)-2-(2-carboxypropan-2-yloxyimino)acetamido)-8-oxo-3-(pyridinium-1-ylmethyl)-5-thia-1-aza-bicyclo[4.2.0]oct-2-ene-2-carboxylate. Its molecular weight is 636.6. The empirical formula is $C_{22}H_{32}N_6O_{12}S_2$.

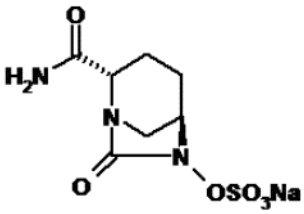
Figure 1: Chemical Structure of Ceftazidime Pentahydrate



- Avibactam

Avibactam sodium chemical name is sodium [(2S,5R)-2-carbamoyl-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl] sulfate. Its molecular weight is 287.23. The empirical formula is $C_7H_{10}N_3O_6SNa$.

Figure 2: Chemical Structure of Avibactam Sodium

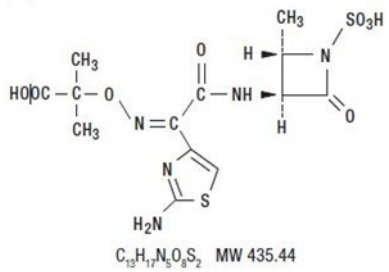


AZACTAM® (aztreonam for injection, USP):

AZACTAM® (aztreonam for injection, USP) injection contains the active ingredient ATM, a monobactam. It was originally isolated from *Chromobacterium violaceum*. It is a synthetic bactericidal antibiotic [18].

The monobactams, having a unique monocyclic beta-lactam nucleus, are structurally different from other beta-lactam antibiotics (e.g., penicillins, cephalosporins, cephamycins). The sulfonic acid substituent in the 1-position of the ring activates the beta-lactam moiety; an aminothiazolyl oxime side chain in the 3-position and a methyl group in the 4-position confer the specific antibacterial spectrum and beta-lactamase stability. Aztreonam is designated chemically as (Z)-2-[[[(2-amino-4-thiazolyl)[[(2S,3S)-2-methyl-4-oxo-1-sulfo-3-azetidinyl]carbamoyl]methylene]amino]oxy]-2-methylpropionic acid.

Figure 3: Chemical Structure of ATM Injection



4.1.1 Formulation, Packaging, and Labeling

AVYCAZ (ceftazidime-avibactam)

AVYCAZ 2.5 grams (2 grams ceftazidime and 0.5 grams avibactam) for injection is a white to yellow sterile powder for constitution consisting of ceftazidime pentahydrate and avibactam sodium packaged in glass vials. The formulation also contains sodium carbonate [19].

Each AVYCAZ 2.5 grams single-dose vial contains ceftazidime 2 grams (equivalent to 2.635 grams sterile ceftazidime pentahydrate/sodium carbonate) and avibactam 0.5 grams (equivalent to 0.551 grams sterile avibactam sodium). The sodium carbonate content of the mixture is 239.6 mg/vial. The total sodium content of the mixture is approximately 146 mg (6.4 mEq)/vial.

AZACTAM® (aztreonam for injection, USP)

AZACTAM (aztreonam for injection, USP) is a sterile, non-pyrogenic, sodium-free, white powder containing approximately 780 mg arginine per gram of ATM. Following constitution, the product is for intramuscular or intravenous use. Aqueous solutions of the product have a pH in the range of 4.5 to 7.5.

The study product vials will be labeled according to manufacturer or regulatory specifications. The dispensed study product (IV bags) will be labeled with the cautionary statement "For Investigational Use Only."

4.1.2 Product Storage and Stability

AVYCAZ (ceftazidime-avibactam)

Storage of AVYCAZ vials:

- AVYCAZ vials should be stored at 20° to 25°C (68° to 77°F); excursions permitted between 15°C and 30°C (59°F and 86°F) [See USP Controlled Room Temperature]. Protect from light. Store in carton until time of use [19].

Stability of AVYCAZ:

- Upon constitution with appropriate diluent, the constituted AVYCAZ solution may be held at room temperature for no longer than 30 minutes prior to transfer and dilution in a suitable infusion bag.
- Following dilution of the constituted solutions with the appropriate diluents, AVYCAZ solutions in the infusion bags are stable for 12 hours when stored at room temperature.
- Following dilution of the constituted solutions with the appropriate diluents, AVYCAZ solutions in the infusion bags may also be refrigerated at 2 to 8°C (36 to 46°F) for up to 24 hours; and then should be used within 12 hours of subsequent storage at room temperature.

AZACTAM® (aztreonam for injection, USP):

Storage of AZACTAM® (aztreonam for injection, USP):

- Store AZACTAM® vials in original packages at room temperature; avoid excessive heat (above 40°C (104° F)).

Stability of Intravenous AZACTAM®:

- AZACTAM solutions for intravenous infusion at concentrations not exceeding 2% weight/ volume (w/v) must be used within 48 hours following constitution if kept at controlled room temperature 15° to 30°C (59° to 86°F) or within 7 days if refrigerated (2° to 8°C/ 36° to 46°F).

4.2 Acquisition/Distribution

AVYCAZ (ceftazidime-avibactam)

AVYCAZ vials manufactured by Allergan will be purchased by the study site's Investigational Pharmacy.

AZACTAM® (aztreonam for injection, USP):

AZACTAM® vials manufactured by Bristol-Myers Squibb will be obtained by the DMID Clinical Materials Services (CMS) and shipped to the following address at Fisher BioServices for storage and site distribution:

DMID-CMS
20439 Seneca Meadows Parkway
Germantown, MD 20876
Tel: 1 (240) 477-1350
Fax: 1 (240) 477-1360
E-mail: DMID.CMS@ThermoFisher.com

AZACTAM® will be shipped to Duke IDS upon request by the study site and approval by DMID.

Sterile Water for Injection (WFI), USP

The sterile water for injection (WFI), USP is nonpyrogenic and contains no bacteriostatic, antimicrobial agent, or added buffer. Each gram of ATM should be initially reconstituted with at least 3 mL WFI, USP. Single-dose container WFI will be purchased by the study sites Investigational Pharmacy.

0.9% Sodium Chloride, USP (Normal Saline)

0.9% Sodium Chloride Injection, USP is a sterile, nonpyrogenic, isotonic solution of sodium chloride and water for injection. Each mL contains 9 mg of sodium chloride and contains no preservatives, bacteriostatic, antimicrobial agent, or added buffer. The solution is clear in appearance and may contain hydrochloric acid and/or sodium hydroxide for pH adjustment (pH 5.3 [4.5 to 7.0]). Normal saline in single use and IV bags will be purchased by the Investigational Pharmacy for dilution of AVYCAZ, preparation of IV administration bags for both study products and flushing of IV lines. The appropriate size IV bag will be determined by the Investigational Pharmacy to ensure the correct amount of study product is being infused.

4.3 Dosage/Regimen, Preparation, Dispensing and Administration of Study Intervention/Study Product

Both AVYCAZ and ATM will be prepared in separate IV bags according to the products' IV infusion directions in the package insert. Complete instructions for dosage, preparation, labeling, storage, stability, and administration is detailed in protocol-specific Manual of Procedures (MOP). Dosing will be completed the morning of Day 7 for all cohorts.

For cohorts 5 and 6, both drugs will be administered either via a double lumen catheter connected to separate ports, or via a single lumen Y-site injection port. Stability data in both the AVYCAZ and ATM package insert and protocol-specific Y-site stability studies support CI administration outlined in Table 1.

The infusion times listed in the table below are approximate (refer to the MOP for infusion time windows). Dose modifications will not be permitted.

Table 1: Treatment Cohorts (8 subjects/cohort)

Cohort 1:	AVYCAZ 2.5 g IV as a 2-hour infusion every 8 hours for 7 days (19 total doses)
Cohort 2:	AVYCAZ 2.5 g IV as a 2-hour infusion x 1, then 0.32 g per hour IV daily as a CI (7.5 g/day) for 7 days (20 total doses, IV bag changes every 8 hours for CI)
Cohort 3:	ATM 2 g IV as a 2-hour infusion every 6 hours for 7 days (25 total doses)
Cohort 4:	ATM 2 g IV as a 2-hour infusion x 1, then 0.33 g per hour IV daily as a CI (8 g/day) for 7 days (14 total doses, IV bag changes every 12 hours for CI)
Cohort 5:	AVYCAZ 2.5 g IV as a 2-hour infusion every 8 hours for 7 days and ATM 1.5 g IV as a 2-hour infusion every 6 hours for 7 days (AVYCAZ 19 total doses; ATM 25 total doses)
Cohort 6*:	AVYCAZ 2.5 g IV as a 2-hour infusion every 8 hours for 7 days and ATM 2 g IV as a 2-hour infusion every 6 hours for 7 days (AVYCAZ 19 total doses; ATM 25 total doses)

*A SMC meeting will be convened after completion of cohort 5 to assess whether the study may proceed to cohort 6.

4.4 Accountability Procedures for the Study Intervention/Study Product(s)

AVYCAZ will be purchased by the study site's Investigational Pharmacy. ATM will be obtained by the DMID CMS and shipped to Duke IDS upon request by the study site and approval by DMID. Once received, AVYCAZ and ATM will be stored in and dispensed by the Investigational Pharmacy. Unused product will be destroyed and documented per institutional procedures.

The FDA requires accounting for the disposition of all study products. The Investigator is responsible for ensuring that a current record of product disposition is maintained and product is dispensed only at an official study site by authorized personnel as required by applicable regulations and guidelines. Records of study product disposition, as required by federal law, consist of the date received at the Investigational Pharmacy, lot number(s), each subject assigned study number, date, lot number(s) and quantity administered to each subject and final disposition for unused study product.

The Investigational Pharmacist will be responsible for maintaining accurate records of the shipment and dispensing of the study product. The pharmacy records must be available for inspection by the Division of Microbiology and Infectious Diseases (DMID) monitoring contractors and is subject to inspection by a regulatory agency (e.g., FDA) at any time. An assigned Study Monitor will review the pharmacy records.

5 SELECTION OF SUBJECTS AND STUDY ENROLLMENT AND WITHDRAWAL

Subject inclusion and exclusion criteria must be confirmed by a study clinician licensed to make medical diagnoses.

No exemptions are granted on subject Inclusion/Exclusion Criteria in DMID-sponsored studies. Questions about eligibility will be directed toward the DMID Medical Officer.

The study site will determine the most efficient procedures to identify potentially eligible subjects from the general public for the study.

5.1 Eligibility Criteria

5.1.1 Subject Inclusion Criteria

A subject must meet all the following criteria to be considered eligible for inclusion in this study:

1. Provide a signed and dated written informed consent.
2. Be able to understand and willing to comply with study procedures, restrictions, and requirements, as determined by the Principal Investigator (PI).
3. Male and female volunteers aged 18 to 45 years inclusive.
4. Suitable veins for cannulation or repeated venipuncture.
5. Subject must be in good general health as judged by the investigator as determined by medical history, vital signs[1], body mass index (BMI) and body weight[2], clinical laboratory values[3], and physical examination (PE).

[1] Oral temp <38.0°C/100.4°F; pulse 50 to 100 bpm; systolic blood pressure 90 to 140 mm Hg, and diastolic blood pressure 55 to 90 mmHg.
[2] BMI between 19-33 kg/m^2 and body weight ≥ 50 kg
[3] Clinical chemistry, hematology, coagulation and urinalysis results within the clinical laboratory reference ranges; clinical laboratory values outside these ranges, if considered by the site investigator to be clinically insignificant, are also acceptable

6. Sexually active female subjects must be of non-childbearing potential[4] or must use a highly effective method of birth control[5].
7. Sexually active male subjects must be vasectomized or agree to use barrier contraception (condom with spermicide) from first dose of study product until 30 days following the last dose of study product.
8. Nonsmokers defined as abstinence from cigarette smoking or use of nicotine-containing products for 6 months prior to enrollment into the study.

5.1.2 Subject Exclusion Criteria

A subject must not meet any of the following criteria to be considered eligible for inclusion in this study:

1. History of any clinically significant (CS) disease or disorder, medical/surgical procedure, or trauma within 4 weeks prior to the first administration of study product(s)[6].
2. History or presence of gastrointestinal, hepatic, or renal disease, or any other condition known to interfere with absorption, distribution, metabolism, or excretion of drugs.
3. Known history of a clinically important allergy/hypersensitivity to AVI, any monobactam, any beta-lactam and/or L-arginine.
4. Receipt of probenecid or furosemide within 14 days prior to study enrollment
5. Receipt of any antibiotics within 14 days prior to study enrollment.
6. 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.

[4] Non-childbearing potential is defined as being post-menopausal for at least 18 months or surgically sterile via hysterectomy, bilateral oophorectomy, or tubal sterilization.
[5] Sexually active female subjects of childbearing potential must avoid becoming pregnant by using one of the following acceptable methods of birth control for 30 days prior to study product dosing and must be maintained for 30 days after last dose of study product:
- Intrauterine contraceptive device; OR
- Approved hormonal contraceptives (such as birth control pills, skin patches, Implanon®, Nexplanon®, DepoProvera®, or NuvaRing®); OR

Birth control must be captured on the appropriate data collection form.
[6] In the opinion of the PI, may either put the volunteer at risk because of participation in the study, or influence the results or the volunteer's ability to participate in the study.

7. Receipt of non-antibiotic medications that interacts with OAT3[7] within 14 days prior to study enrollment.
8. Receipt of herbal and dietary supplements (including St. John's Wort) within 14 days prior to study enrollment.
9. ALT or AST laboratory value above the ULN as defined in the toxicity table in Appendix B.
10. Prolonged QTcF (> 450 msec) or shortened QTcF (< 340 msec) or family history of long QT syndrome. Any clinically important abnormalities in rhythm, conduction, or morphology of resting ECG.[8]
11. Any positive result on screening for human immunodeficiency virus (HIV) serum hepatitis B surface antigen (HBsAg) and hepatitis C virus (HCV) antibody.
12. Creatinine clearance equal or less than 80 mL/minute (measured by Cockcroft-Gault method) [23].
13. History of *Clostridium difficile* infection in past 90 days.
14. Known or suspected history of drug or alcohol abuse within the last 5 years, as judged by the PI.
15. Positive screen for drugs of abuse, cotinine (nicotine), or alcohol at screening and at admission to the study site prior to the first administration of the study products(s).
16. Received a new chemical entity (compound not approved for marketing) or participated in a study that included drug treatment within 1 month of the first dose of study product(s) for study.[9]

    Note: subjects consented and screened, but not dosed in this study or a previous Phase I study will not be excluded.
17. Previous participation in the present study.
18. Involvement in the planning and/or conduct of the study.

[7] 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
[8] Abnormalities that may interfere with interpretation of QTc interval changes per the medical judgment of the PI.
[9] Period of exclusion begins at the time of the last visit of the prior study.

19. Any ongoing/recent (during screening) medical complaints that may interfere with analysis of study data or are considered unlikely to comply with study procedures, restrictions, and requirements.[10]

20. Known history of past or current epilepsy or seizure disorders, excluding febrile seizures of childhood.

5.2 Withdrawal from the Study, Discontinuation of Study Product, or Study Termination

5.2.1 Withdrawal from the Study or Discontinuation of the Study Product

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

An investigator may also withdraw a subject from receiving the study product for any reason. If the subject agrees, follow-up safety evaluations will be conducted. If a subject withdraws or is withdrawn prior to completion of the study, the reason for this decision must be recorded in the case report forms (CRFs).

The reasons, might include, but are not limited to the following:

- Subject withdraws consent
- Subject no longer meets eligibility criteria
- Subject meets individual halting criteria (see Section 8.6.2)
- Subject becomes noncompliant
- Medical disease or condition or new clinical finding(s) for which continued participation, in the opinion of the investigator might compromise the safety of the subject, interfere with the subject's successful completion of this study, or interfere with the evaluation of responses
- Subject lost to follow-up

The investigator should be explicit regarding study follow-up (e.g. safety follow-up) that might be carried out despite the fact the subject will not receive further study product. If the subject consents, every attempt will be made to follow all AEs through resolution. The procedures that collect safety data for the purposes of research must be inclusive in the original informed consent

[10] Judgment by the PI that the subject should not participate in the study.

or the investigator may seek subsequent informed consent using an IRB/IEC-approved consent form with the revised procedures.

Every attempt should be made to complete a physical examination and all Day 8 procedures for all subjects who withdraw from the study or discontinue study product.

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

5.2.2 Subject Replacement

Subjects who are enrolled but drop out before receiving study product(s) will be replaced. If two or more subjects in a dosing cohort have an incomplete PK profile, subjects will be replaced in that dosing cohort to ensure there are at least seven subjects with complete PK profiles in each dosing cohort. 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.

5.2.3 Study Termination

If the study is prematurely terminated by the sponsor, any regulatory authority, or the investigator for any reason, the investigator will promptly inform the study subjects and assure appropriate therapy or follow-up for the subjects, as necessary (see Section 6.4 and 8.3). The investigator will provide a detailed written explanation of the termination to the IRB/IEC.

6 STUDY PROCEDURES

The study schedule is outlined below and in the Schedule of Events (Appendix A). Protocol waivers or exemptions are not allowed. Therefore, adherence to the study design requirements, including those specified in the Schedule of Events, is required.

Amendments or changes to the protocol require the approval of the sponsor and the Institution Review Board (IRB).

6.1 Screening (Days -30 to Day -2)

The study site will determine the most efficient procedures to identify potentially eligible subjects for the study. Subjects will report to the study site between Days -30 to Day -2. The study will be explained to each potential subject. A signed and dated IRB-approved informed consent form (ICF) must be properly obtained from each subject before any study-specific test or evaluation is administered. Approximately 75 subjects will be screened to enroll 48 evaluable subjects. Individuals who agree to participate and sign the informed consent will undergo the following procedures [also see Schedule of Events (Appendix A)].

- Obtain informed consent
- Review and obtain medical history, concomitant medication(s), demographics, height, and weight. Ensure review of any history of hypersensitivity to drugs with a similar chemical structure or class to ATM, AVI or CAZ.
- Complete physical examination
- Vital signs: Systolic and diastolic blood pressures (mmHg), pulse rate (BPM), respiratory rate, and body temperature (oral measurement)
- 12-lead ECG in triplicate (mean value of ECGs will be used to determine study eligibility)
- Clinical chemistry: sodium (Na), potassium (K), chloride (Cl), bicarbonate (HCO_3), blood urea nitrogen (BUN), serum creatinine, glucose, albumin, total protein, total bilirubin, ALT, AST, ALP and lactate dehydrogenase (LDH)
- Hematology: Hemoglobin, hematocrit, white blood cell count (WBC), WBC differential, red blood cell count (RBC), platelets
- Coagulation: PT and Partial thromboplastin time (PTT)
- Viral serology: HIV, HBsAg, HCV antibody
- Coombs test

- Urinalysis: dipstick urinalysis, including protein, glucose, ketones, bilirubin, blood, nitrites, leukocyte esterase (LCE), urobilinogen, specific gravity, and pH.

  NOTE: The administration of ceftazidime may result in a false-positive reaction for glucose in the urine with certain methods. The clinical laboratory equipment will measure glucose via glucose oxidase reactions, which is not affected by ceftazidime administration. [17]

- Urine drug screening
- Pregnancy testing (females of child bearing potential only): Serum human chorionic gonadotropin (hCG)
- Alcohol Screen Breathalyzer
- Cotinine Test

6.2 Enrollment (Day -1)

Subjects will report to the study site on Day -1 for the procedures listed below and in the Schedule of Events (Appendix A). Once eligibility has been confirmed, subjects will be enrolled into the study and assigned to a treatment cohort. Assessments at Day -1 will serve as baseline values. Subjects will remain at the study site until discharge on Day 8 or until withdrawal from the study (see Section 5.2).

- Complete physical examination, height and weight, concomitant medication review and review for changes in medical history
- Vital signs: Systolic and diastolic blood pressures (mmHg), pulse rate (BPM), respiratory rate, and body temperature (oral measurement)
- Single 12-lead ECG
- Clinical chemistry: Na, K, Cl, HCO_3, BUN, serum creatinine, glucose, albumin, total protein, total bilirubin, ALT, AST, ALP and LDH
- Hematology: Hemoglobin, hematocrit, WBC, WBC differential, RBC, platelets
- Coagulation: PT and PTT
- Urinalysis and urine drug screening
- Pregnancy testing (females of child bearing potential only): Serum hCG if not already performed within 48 hours of Day -1. Results must be available prior to administration of study product.

- Alcohol Screen Breathalyzer
- Cotinine Test

6.3 Planned Study Visits (Days 1 – 8)

6.3.1 Treatment (Day 1)

The following procedures will be completed as listed below and in the Schedule of Events (Appendix A):

- Symptom directed physical exam
- Vital signs will be obtained prior to dosing on Day 1 and 1 hour after starting AM dose
- Monitoring for AEs will begin after the first dose of study product
- Review for any new medication
- Study product administration as outlined in Section 4
- Plasma and urine PK as outlined in Sections 7.2.2.

6.3.2 Treatment (Day 2)

The following procedures will be completed as listed below and in the Schedule of Events (Appendix A):

- Symptom directed physical exam
- Vital signs will be obtained in the morning
- Monitor for AEs.
- Review for any new medication
- Study product administration as outlined in Section 4.
- Clinical chemistry: Na, K, Cl, HCO_3, BUN, serum creatinine, glucose, albumin, total protein, total bilirubin, ALT, AST, ALP and LDH
- Hematology: hemoglobin, hematocrit, WBC, WBC differential, RBC, platelets
- Coagulation: PT and PTT

6.3.3 Treatment (Day 4, 6)

The following procedures will be completed as listed below and in the Schedule of Events (Appendix A):

- Symptom directed physical exam
- Vital signs will be obtained in the morning
- Monitor for AEs.
- Review for any new medication
- Study product administration as outlined in Section 4.
- Clinical chemistry: Na, K, Cl, HCO_3, BUN, serum creatinine, glucose, albumin, total protein, total bilirubin, ALT, AST, ALP and LDH
- Hematology: hemoglobin, hematocrit, WBC, WBC differential, RBC, platelets
- Coagulation: PT and PTT
- **Day 4 only**: Urinalysis
- **Day 6 only**: Urine PK as outlined in Section 7.2.2.2

6.3.4 Treatment (Day 3, 5, 7)

The following procedures will be completed as listed below and in the Schedule of Events (Appendix A):

- Symptom directed physical exam
- Vital signs will be obtained in the morning
- Monitor for AEs
- Review for any new medication
- Study product administration as outlined in Section 4.
- Plasma PK as outlined in Section 7.2.2.1
- **Day 5 and 7 only:** LFTs: ALT, AST, ALP, and total bilirubin

6.3.5 Discharge (Day 8)

The following procedures will be completed as listed below and in the Schedule of Events (Appendix A):

- Complete physical examination, concomitant medication review and review for changes in medical history
- Vital signs will be obtained in the morning
- Single 12-lead ECG
- Assessment of AEs
- Clinical chemistry: Na, K, Cl, HCO_3, BUN, serum creatinine, glucose, albumin, total protein, total bilirubin, ALT, AST, ALP and LDH
- Hematology: Hemoglobin, hematocrit, WBC, WBC differential, RBC, platelets
- Coagulation: PT and PTT
- Coombs Test
- Urinalysis
- Plasma PK as outlined in Section 7.2.2.1
- Schedule Day 11 Final Study Visit

6.3.6 Final Study Visit (Day 11 +3)

Subjects will report to the study site on Day 11 + 3 for the final study visit and completion of the assessments as listed below and in the Schedule of Events (Appendix A):

- Complete physical examination and concomitant medication review
- Vital signs
- Single 12-lead ECG
- Assessment of AEs
- Clinical chemistry: Na, K, Cl, HCO_3, BUN, serum creatinine, glucose, albumin, total protein, total bilirubin, ALT, AST, ALP and LDH
- Hematology: Hemoglobin, hematocrit, WBC, WBC differential, RBC, platelets
- Coagulation: PT and PTT
- Urinalysis

6.4 Early Termination Visit

- In circumstances where a subject discontinues the study early, an early termination visit will be performed. Every attempt should be made to complete a physical examination and all other Day 8 procedures for all subjects who withdraw from the study or discontinue study product. The reason(s) for early termination should be reflected in the source documentation and the Study Termination electronic case report form (eCRF). Complete physical examination, concomitant medication review and review for changes in medical history
- Vital signs
- Single 12-lead EGC
- Assessment of AEs
- Clinical chemistry: Na, K, Cl, HCO_3, BUN, serum creatinine, glucose, albumin, total protein, total bilirubin, ALT, AST, ALP and LDH
- Hematology: Hemoglobin, hematocrit, WBC, WBC differential, RBC, platelets
- Coagulation: PT and PTT
- Coombs Test
- Urinalysis
- Sample for a single plasma PK level

6.5 Unscheduled Study Visits

The PI may decide a subject requires an unscheduled visit outside the normal schedule. At a minimum this visit will include a physical exam, clinical chemistry, hematology, coagulation test, and AE assessment. However the following may be included based on the judgement of the PI.

- Complete physical examination, concomitant medication review and review for changes in medical history
- Vital signs
- Single 12-lead ECG
- Assessment of AEs
- Clinical chemistry: Na, K, Cl, HCO_3, BUN, serum creatinine, glucose, albumin, total protein, total bilirubin, ALT, AST, ALP and LDH

- Hematology: Hemoglobin, hematocrit, WBC, WBC differential, RBC, platelets
- Coagulation: PT and PTT
- Coombs Test
- Urinalysis
- Sample for a single plasma PK level (optional)

Unscheduled study visits will be assigned a trailing alpha letter after the study day number. For example, if a subject has an unscheduled study day that occurs between study day 1 and study day 2, the study visit day would be numbered as study day 1S.

6.6 Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol, GCP, or protocol-specific MOP requirements. The noncompliance may be either on the part of the subject, the site PI, or the site personnel. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

These practices are consistent with ICH E6:

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

5.1 Quality Assurance (QA) and Quality Control (QC), Section 5.1.1

5.20 Noncompliance, Sections 5.20.1, and 5.20.2.

It is the responsibility of the site PI and personnel to use continuous vigilance to identify and report deviations within five working days of identification of the protocol deviation, or within five working days of the scheduled protocol-required activity. All deviations must be promptly reported to DMID per the Statistical and Data Coordinating Center (SDCC) protocol deviation reporting procedures.

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

7 DESCRIPTION OF CLINICAL AND LABORATORY EVALUATIONS

7.1 Clinical Evaluations

7.1.1 Research Procedures

The following will be obtained:

- Complete medical history – subjects will be queried regarding a history of significant medical disorders and any allergies. Specifically querying if the subject has a history of hypersensitivity to drugs with a similar chemical structure or class to ATM, AVI or CAZ.
- Complete medications history – subjects will be queried regarding all prescription and over-the-counter medications and supplements taken within 24 hours and any study products taken within 90 days prior to start of study products.
- A complete physical examination should include general appearance, neurological, head, eye, ear, nose, throat (HEENT), cardiovascular, lungs, abdomen, musculoskeletal, and skin examinations. The physical examination performed at Day -1 will serve as the baseline for clinical assessments.
- A targeted or focused physical examination is symptom directed and includes neurological, HEENT, and musculoskeletal examinations and is specifically related to reported AEs during the study (e.g., abdomen for AEs of vomiting or diarrhea).
- Height and weight will be measured at Screening and Day -1 to calculate the BMI.
- Vital signs should be collected after resting in supine position. Vital signs include systolic and diastolic blood pressures (mmHg), pulse rate (BPM), respiratory rate, and body temperature.
- The PI or his designee will interpret ECGs in real time during inpatient dosing periods and within 12 hours for late-night tracings. If a CS abnormality is detected, an appointed cardiologist at the study site will immediately interpret the ECG. A licensed physician listed on Form FDA 1572 will review the final results. Subjects with CS findings will be referred either to the emergency department or to their primary care physician for follow-up.

  An ECG will be recorded in triplicate approximately 2 minutes apart at the Screening visit. Single ECGs will be recorded on Days -1, 8, and 11 (+3). Subjects must be lying supine or sitting for 10 minutes before recording the ECG tracings.

The ECGs will be printed and evaluated by an appropriately qualified physician at the study site. The reader will sign and date the safety ECGs and provide a global interpretation using the following categories:

- Normal ECG
- Abnormal ECG-not clinically significant (NCS)
- Abnormal ECG-CS
- Unable to evaluate
- Other -details will be noted in the source document and eCRF

7.1.2 Assessment of Concomitant Medications/Treatments Other Than Study Product

Use of concomitant medications (probenecid and furosemide) that are contraindicated in the AVYCAZ and ATM package inserts will not be permitted [18,19]. Subjects taking such medications will not be eligible for the study.

Patients who received antibiotics within the 14 days prior to study enrollment will not be eligible for the study.

Patients receiving a non-antibiotic medication that interacts with OAT3 (e.g., 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) will not be included [30,31].

In addition, no prescription (except birth control pills or hormone replacement in females) or non-prescription drugs will be permitted, unless in the opinion of site investigator the medication will not interfere with the study procedures or impact subject safety. The use of herbal and dietary supplements (including St. John's Wort) will not be permitted during the study.

7.1.3 Assessment of Subject Compliance With the Study Visit Schedule

The Investigational Pharmacist or designee is responsible for the reconstitution and labeling of the study products. Study products will be administered via IV infusion by a member of the clinical research team who is licensed to administer the study products. Administration will be documented on the source document and entered into the eCRF.

Study product compliance including start and stop time of infusion, volume administered, and the occurrence of any infusion interruptions (greater than 10 minutes) will be captured in the source documents.

7.2 Laboratory Evaluations

Subjects will have up to approximately 135 mL of blood drawn for safety laboratory tests in each of the study cohorts.

Safety laboratory monitoring will include: hematology, clinical chemistry, LFTs, coagulation, viral serology and urinalysis.

7.2.1 Clinical Laboratory Evaluations

- Clinical chemistry: Na, K, Cl, HCO_3, BUN, serum creatinine, glucose, albumin, total protein, total bilirubin, ALT, AST, ALP and LDH
- LFTs: ALT, AST, ALP, and total bilirubin
- Hematology: Hemoglobin, hematocrit, WBC, WBC differential, RBC, platelets
- Coombs Test
- Coagulation: PT, PTT
- Serology: HIV, HBsAg, HCV
- Urinalysis: dipstick urinalysis, including protein, glucose, ketones, bilirubin, blood, nitrites, LCE, urobilinogen, specific gravity, and pH. The administration of ceftazidime may result in a false-positive reaction for glucose in the urine with certain methods. The clinical laboratory equipment will measure glucose via glucose oxidase reactions, which is not affected by ceftazidime administration.
- Pregnancy testing (females of child bearing potential only): Serum human chorionic gonadotropin (hCG).
- Alcohol screen breathalyzer
- Cotinine Test
- Urine drug screen: amphetamines, barbiturates, benzodiazepines, cocaine metabolites, opiates, cannabinoids and phencyclidine

7.2.2 Research Assays

7.2.2.1 Plasma Pharmacokinetic Sample Collection

Venous plasma samples for pharmacokinetic analyses of AVYCAZ and ATM will be collected at the following time points ±5 minutes for pre-dose, during, and end of short infusion samples and then ±15 minutes for other samples. The actual date and time of each blood sample collection will be recorded in the subjects' source document and the eCRF. The timing of the study assessments should allow the blood draw to occur at the exact nominal time.

Approximately 192 mL of blood will be obtained from each subject for determining plasma AVYCAZ and ATM for each dosing regimen. Blood collected will be processed at the study site's laboratory.

Cohort 1 (AVYCAZ Q8 hours treatment cohort)

- Day 1: blood samples will be collected pre-dose (10 minutes prior to the start of 1st infusion), 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, and 8 hours after the start of the 1st infusion (8 hour sample **must be** collected prior to the start of the 2nd dose of AVYCAZ).
- Day 3: Blood samples will be collected 10 minutes prior to the start of the 1st infusion on Day 3.
- Day 5: Blood samples will be collected 10 minutes prior to the start of the 1st infusion on Day 5.
- Day 7, blood samples will be collected pre-dose (10 minutes prior to the start of the morning infusion on Day 7, which is the last infusion of the study), 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8, 10, 12, 18, and 24 hours after the start of the Day 7 infusion.

Cohorts 2, 4 (CI treatment cohort)

- Day 1: blood samples will be collected at pre-dose (10 minutes prior to the start of 1st infusion), 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, and 8 hours after the start of the 1st infusion of Day 1.
- Day 3: Blood samples will be collected 10 minutes prior to the start of the 1st infusion on Day 3.
- Day 5: Blood samples will be collected 10 minutes prior to the start of the 1st infusion on Day 5.
- Day 7: Blood samples will be collected 10 minutes prior to the start of the 1st infusion(s) on Day 7 (last infusion(s) of the study), 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8, 10, 12, 18, and 24 hours after the start of the Day 7 infusion(s).

Cohort 3 (ATM Q6 hours treatment cohort)

- Day 1: blood samples will be collected pre-dose (10 minutes prior to the start of 1st infusion), 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6 (6 hour sample **must be** collected prior to the start of the 2nd dose of ATM), 7, and 8 hours after the start of the 1st infusion.
- Day 3: Blood samples will be collected 10 minutes prior to the start of the 1st infusion on Day 3.
- Day 5: Blood samples will be collected 10 minutes prior to the start of the 1st infusion on Day 5.
- Day 7: blood samples will be collected pre-dose (10 minutes prior to the start of the morning infusion on Day 7, which is the last infusion of the study), 0.5, 1, 1.5, 2 , 2.5, 3, 4, 5, 6, 7, 8, 10, 12, 18, and 24 hours after the start of the Day 7 infusion.

Cohort 5, 6 (AVYCAZ Q8 hours + ATM Q6 hours treatment cohort)

- Day 1: blood samples will be collected pre-dose (10 minutes prior to the start of 1st infusion), 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6 (6 hour sample **must be** collected prior to the start of the 2nd dose of ATM), 7, and 8 hours after the start of the 1st infusion (8 hour sample **must be** collected prior to the start of the 2nd dose of AVYCAZ).
- Day 3: Blood samples will be collected 10 minutes prior to the start of the 1st infusion on Day 3.
- Day 5: Blood samples will be collected 10 minutes prior to the start of the 1st infusion on Day 5.
- Day 7: blood samples will be collected pre-dose (10 minutes prior to the start of the morning infusion on Day 7, which is the last infusion of the study), 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8, 10, 12, 18, and 24 hours after the start of the Day 7 infusion.

7.2.2.2 Urine Pharmacokinetic Sample Collection

Each study subject will have serial urine samples collected on Day 1 and Day 6 to determine urine concentrations of AVYCAZ and ATM alone and in combination. The actual date and time of each urine sample collection will be recorded in the subjects' source document and the eCRF. The total volume of urine for each collection interval will be recorded. After measuring total volume of urine for each collection interval, aliquots of urine samples will be taken from the collected urine sample and transferred into suitably labeled polypropylene cryogenic sample storage vials and maintain being chilled before being transferred to freezer.

Days 1 and 6:

Serial urine samples will be collected for all cohorts at the following intervals: >0 to 4, >4 to 8, >8 to 12, and >12 to 24 hours after the start of the morning dose administration(s) of the day.

7.2.2.3 Laboratory Specimen Preparation, Handling, and Storage

Plasma and urine PK specimens will be processed and stored in a -80^{o}C freezer until time of shipment to Keystone Bioanalytical laboratory for analysis.

Detailed instructions for the preparation, handling, and storage of plasma and urine PK specimens are detailed in the study MOP including aliquots of specimens, temperature requirements, where they will be stored, and how they will be labeled.

7.2.2.4 Laboratory Specimen Shipping

After the completion of each dosing cohort, both plasma and urine PK samples for all subjects in each cohort will be shipped on dry ice to Keystone Bioanalytical laboratory for analysis. Samples will be shipped in compliance with the International Air Transport Association (IATA) regulations. Plasma and urine PK samples will be shipped in separate shipping containers. Instructions for the shipment of specimens are outlined in the study MOP.

8 ASSESSMENT OF SAFETY

8.1 Assessing and Recording Safety Parameters

This study will assist in determining the safety profile for the combination of AVYCAZ with ATM, AVYCAZ alone and ATM alone. The same safety data will be collected across all treatment cohorts. AVYCAZ and ATM both are approved drugs with established safety profiles. However, the AVYCAZ CI regimens examined as part of this study are not FDA approved. There is also limited published safety data on ATM alone.

To ensure a clear comparison of safety between the single dose cohorts and the combination dosing cohorts for this study, AEs will be collected for all study cohorts.

8.1.1 Adverse Events (AEs)

Monitoring for AEs will begin after starting infusion of the first dose of study product on Day 1 through the final Study Visit on Day 11 +3.

ICH E6 defines an AE as any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product regardless of its causal relationship to the study treatment. FDA defines an AE as any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.

An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of medicinal (investigational) product. The occurrence of an AE may come to the attention of study personnel during study visits and interviews of a study recipient presenting for medical care, or upon review by a study monitor.

All AEs, including local (infusion site) and systemic reactions, will be captured on the appropriate data collection form and eCRF. Information to be collected for AEs includes event description, date of onset, assessment of severity, relationship to study product and alternate etiology (assessed only by those with the training and authority to make a diagnosis and listed on the Form FDA 1572 as an investigator), date of resolution, seriousness and outcome. AEs occurring during the trial collection and reporting period will be documented appropriately regardless of relationship. AEs will be followed through resolution, return to pretreatment status or stabilization of the condition with the expectation that it will remain chronic.

Any medical condition that is present at the time that the subject is screened will be considered as baseline and not reported as an AE. However, if the severity of any pre-existing medical condition worsens, it should be recorded as an AE.

8.1.1.1 Adverse Events Grading

All AEs (laboratory and clinical symptoms) will be graded for severity (Appendix B) and assessed for relationship to study product (see definitions below). AEs characterized as intermittent require documentation of onset and duration of each episode. The start and stop date of each reported AE will be recorded on the appropriate data collection form and eCRF. Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of intensity.

Severity of Event:

AEs will be assessed by the investigator using a protocol-defined grading system (Laboratory and Vital Signs Reference Ranges, Eligibility Ranges, and Toxicity Grading information in Appendix B). For events not included in the protocol-defined grading system, the following guidelines will be used to quantify severity:

- Mild (Grade 1): Events that are usually transient (less than 48 hours) and may require only minimal or no treatment or therapeutic intervention and generally do not interfere with the subject's usual activities of daily living.
- Moderate (Grade 2): Events that are usually alleviated with additional specific therapeutic intervention (use of non-narcotic pain reliever or over-the-counter medication). The event interferes with usual activities of daily living, causing discomfort but poses no significant or permanent risk of harm to the research participant.
- Severe (Grade 3): Events interrupt usual activities of daily living, or significantly affects clinical status, or may require intensive therapeutic intervention (requires prescription medication, or IV fluids or medical procedure). Severe events are usually incapacitating.

Relationship to Study Product: The assessment of the AE's relationship to study product will be done by the licensed study physician indicated on the Form FDA 1572 and the assessment will be part of the documentation process. Whether the AE is related or not to the study product, is not a factor in determining what is or is not recorded in the eCRF in this trial. If there is any doubt as to whether a clinical observation is an AE, the event should be recorded in the eCRF.

In a clinical trial, the study product must always be suspect. The relationship to study product will be assessed for AEs using the terms related or not related:

- Related – There is a reasonable possibility that the study product caused the AE. Reasonable possibility means that there is evidence to suggest a causal relationship between the study product and the AE.
- Not Related – There is not a reasonable possibility that the administration of the study product caused the event.

8.1.2 Serious Adverse Events (SAEs)

An AE is considered an SAE if, in the view of either the site PI or sponsor, it results in any of the following outcomes:

- Death,
- a life-threatening AE[11],
- inpatient hospitalization or prolongation of existing hospitalization,
- a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or
- a congenital anomaly/birth defect,
- Important medical events that may not result in death, be life-threatening, or require hospitalizations may be considered serious when, based upon appropriate medical judgment they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

All SAEs will be:

- Assessed for severity and relationship to study product and alternate etiology (if not related to study product) by a licensed study physician listed on the Form FDA 1572 or by the Institution as the site PI or Sub-Investigator.
- Recorded on the appropriate SAE data collection form and eCRF.

Followed through resolution or stabilization, even if this extends beyond the study-reporting period, by a licensed study physician (for Investigational New Drug Application (IND) studies, a physician listed on the Form FDA 1572 as the site PI or Sub-Investigator).

- Reviewed and evaluated by DMID, an Independent Safety Monitor (ISM) (as deemed necessary) or SMC (periodic review unless related), and the IRB/IEC.

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

8.2 Specification of Safety Parameters

8.2.1 Solicited Events

Solicited events will not be collected as part of this study.

8.2.2 Unsolicited Events

Unsolicited events are AEs that occur following administration of study product. Subjects can be queried as needed to evaluate occurrence of any AEs.

8.3 Reporting Procedures

8.3.1 Reporting Serious Adverse Events

For this study, all AE/SAEs occurring from first dose of study product on Day 1 through Day 11 + 3 days after last dose of study product(s) will be collected in the eCRF.

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

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

In addition to the SAE form, select SAE data fields must also be entered into the SDCC system that must match the data on the SAE Report form. Please see the protocol-specific MOP for details regarding this procedure.

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

The site will send a copy of the SAE report(s) to the ISM when they are provided to the DMID Pharmacovigilance Group. The DMID Medical Monitor (MM) and DMID Clinical Project Manager will be notified of the SAE by the DMID Pharmacovigilance Group. The DMID Medical Monitor will review and assess the SAE for regulatory reporting and potential impact on study subject safety and protocol conduct.

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

8.3.2 Regulatory Reporting for Studies Conducted Under DMID-Sponsored IND

Following notification from the site PI or appropriate sub-investigator, DMID, as the IND sponsor, will report any suspected unexpected serious adverse event (SUSAR). DMID will report an SAE as a SUSAR only if there is evidence to suggest a causal relationship between the study intervention and the SAE. DMID will submit an IND safety report to the FDA and will notify all participating site PIs (i.e., all PIs to whom the sponsor is providing drug under its IND(s) or under any PI's IND(s)of potential serious risks from clinical studies or any other source, as soon as possible. DMID will report to the FDA any unexpected fatal or life-threatening suspected adverse reaction as soon as possible, but in no case later than seven (7) calendar days after the sponsor's initial receipt of the information. If the event is not fatal or life-threatening the IND safety report will be submitted within 15 calendar days after the sponsor determines that the information qualifies for reporting as specified in 21 CFR Part 312.32. Relevant follow up information to an IND safety report will be submitted as soon as the information is available. Upon request from FDA, DMID will submit to the FDA any additional data or information that the agency deems necessary, as soon as possible, but in no case later than 15 calendar days after receiving the request.

All SAEs designated as "not related" to study product(s), will be reported to the FDA at least annually in a summary format.

8.3.3 Reporting of Pregnancy

Female subjects of childbearing age who become pregnant during the study will be discontinued from study medication and will be followed for pregnancy outcome. Pregnancy occurring during a clinical investigation, although not considered a SAE, must be reported within the same timelines as a SAE. The positive pregnancy test will be recorded in the database within 5 days of site awareness, on the Pregnancy Report form. The report will include pregnancy outcome (e.g., any premature terminations, elective or therapeutic, any spontaneous abortions or stillbirths), as well as the health status of the mother and child, including date of delivery and infant's sex and weight. Any subject with a positive pregnancy test, who has received study product(s), will be followed through eight (8) weeks post-live delivery or elective or natural termination of the pregnancy, whichever occurs first. If the database is locked at time of pregnancy, a supplemental report will be generated and completed after birth, which will be appended to the database. Any occurring AEs or SAEs that occur to the mother or fetus will be recorded in the eCRF in the database and on the SAE Report form.

The site is responsible for notifying their local IRB of any pregnancies in accordance with local policies.

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

Adverse events, including serious AEs, will be assessed and collected from initial recognition of the AE occurring from first dose of study product on Day 1 through end of the protocol defined follow-up period (Day 11 + 3).

All AEs and serious AEs occurring from first dose of study product on Day 1 through Day 11 + 3 days will be followed up through resolution even if duration of follow-up goes beyond the protocol-defined follow-up period.

Resolution of an AE is defined as the return to pre-treatment status or stabilization of the condition with the expectation that it will remain chronic.

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

Mild abnormal laboratory values (Grade 1) that are not, in the investigator's opinion, medically significant should not be reported as AEs. Any Grade 2 or higher abnormal laboratory value (see Appendix B) will be reported as an AE regardless of the relationship to the study product. Laboratory abnormalities reported as AEs will be followed until the abnormality stabilizes or normalizes.

8.6 Halting Rules

8.6.1 Study Halting Criteria

If any of the following events occur, enrollment and dosing for all subject will be suspended until the event is assessed by the SMC:

- Any subject develops an SAE related to the study product through the last study visit.
- In cohorts 2, 4, 5, and 6, two (2) or more subjects, in a single treatment cohort, experience a Grade 3 (severe) AE (including clinical, ECG, vital signs and laboratory AEs) that is related to study product and is of the same type [High Level Term (HLT)].
- In cohorts 5 and 6, three (3) or more subjects (cumulative) experience a Grade 3 (severe) AE (including clinical, ECG, vital, and laboratory AEs) that is related to the study products (combination) and is of the same type (HLT).
- Any subject develops anaphylaxis within 24 hours after receiving the study product.

8.6.2 Individual Halting Criteria

Further dosing of study product will be halted for any individual subject if any one of the following occurs:

- Any SAE related to the study product.
- Any Grade 3 AE event related to the study product.
- Any other condition that the study site PI judges to unduly increase the risk to the subject.

8.7 Safety Oversight

8.7.1 Independent Safety Monitor (ISM)

For this clinical trial, DMID will require an Independent Safety Monitor (ISM) to be assigned for each study site and the requirement for an ISM will be specified in the protocol. An ISM is a physician with relevant expertise whose primary responsibility is to provide to DMID an independent safety assessment in a timely fashion. The ISM will review all SAEs in real time and other AEs as needed and provide an independent assessment to DMID.

8.7.2 Safety Monitoring Committee (SMC)

This clinical study will utilize a SMC, which is an independent group of experts that advises the DMID. The primary responsibility of the SMC is to monitor subject safety. The SMC is external to the DMID and comprises at least 3 voting members. The SMC will consist of members with appropriate phase 1 study expertise to contribute to the interpretation of the data from this trial. Its activities will be delineated in an SMC charter that will describe the membership, responsibilities, and the scope and frequency of data reviews. The SMC will operate on a conflict-free basis independently of the study team. The DMID or the SMC may convene ad hoc meetings of the SMC according to protocol criteria or if there are concerns that arise during the study.

As defined in the charter, the SMC will review data at specified times during the course of the study for subject and overall study progress, and may conduct ad hoc reviews as appropriate when a halting rule is met or for immediate concerns regarding observations during this study.

The SMC will meet as follows:

- Organization meeting (prior to start of the study).
- Ad-hoc meeting when a halting rule is met or by request of the PI, ISM, or DMID MM if any safety concern arises.
- Upon completion of cohort 5, prior to enrolling cohort 6.

- Final review meeting, to be conducted 6 to 8 months after clinical database lock to review the cumulative safety data for the study.

The data will be provided in a standard summary format. The SMC may be asked to provide recommendations in response to questions posed by the DMID.

Study product(s) administration data, including dose interruptions, modifications, and the associated reason(s), will be reported to the SMC.

The SMC will operate under the rules of a DMID-approved charter. Data reviews may include enrollment and demographic information, medical history, concomitant medications, physical assessments, dosing compliance, protocol adherence, clinical laboratory values, PK data, and SAEs. Additional data may be requested by the SMC, and interim statistical reports may be generated as deemed necessary and appropriate by DMID. The SMC will receive data in aggregate and stratified by treatment cohort. The objective of the SMC is to make recommendations to the sponsors if the study should continue per protocol, be modified and then proceed, or be terminated. After each review/meeting the SMC will make recommendations as to the advisability of proceeding with study (as applicable), and to continue, modify, or terminate this study.

The DMID Medical Monitor or Medical Officer is empowered to stop study enrollment and administration if halting criteria are met or if any serious safety concerns arise.

9 HUMAN SUBJECTS PROTECTION

9.1 Institutional Review Board/Independent Ethics Committee

Each site PI will obtain IRB approval for this protocol to be conducted at his/her research site(s), and send supporting documentation to the DMID before initiating recruitment of subjects. The investigator will submit applicable information to the IRB/IEC on which it relies for the review, to conduct the review in accordance with 45 CFR 46, ICH E6 GCP, and as applicable, 21 CFR 56 (Institutional Review Boards) and 21 CFR 50 (Protection of Human Subjects), other federal, state, and local regulations. The IRB/IEC must be registered with OHRP as applicable to the research. DMID must receive the documentation that verifies IRB/IEC-approval for this protocol, associated informed consent documents, and upon request any recruitment material and handouts or surveys intended for the subjects, prior to the recruitment and enrollment of subjects.

Any amendments to the protocol or consent materials will be approved by the IRB/IEC before they are implemented. IRB/IEC review and approval will occur at least annually throughout the enrollment and follow-up of subjects, and may cease if annual review is no longer required by applicable regulations and the IRB/IEC. The investigator will notify the IRB/IEC of deviations from the protocol and reportable SAEs, as applicable to the IRB/IEC policy.

Each institution engaged in this research will hold a current Federalwide Assurance (FWA) issued by the Office of Human Research Protection (OHRP) for federally funded research.

9.2 Informed Consent Process

Informed consent is a process that is initiated prior to an individual agreeing to participate in a clinical trial and continuing throughout the individual's clinical trial participation. Before any study procedures are performed, informed consent will be obtained and documented. Subjects will receive a concise and focused presentation of key information about the clinical trial, verbally and with a written consent form. The explanation will be organized and presented in lay terminology and language that facilitates understanding why one might or might not want to participate.

An investigator or designee will describe the protocol to potential subjects face-to-face. The key information about the purpose of the study, the procedures and experimental aspects of the study, risks and discomforts, any expected benefits to the subject, and alternative treatment will be presented first to the subject.

Subjects will also receive an explanation that the trial involves research, and a detailed summary of the proposed study procedures and study interventions/products. This will include aspects of

the trial that are experimental, the probability for random assignment to treatment cohort, 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, alternative procedures that may be available and the important potential benefits and risks of these available alternative procedures.

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. 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. The subjects will be informed that participation is voluntary and that they are free to withdraw from the study for any reason at any time without penalty or loss of benefits to which the subject is otherwise entitled.

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), auditors(s), IRB, NIAID, and regulatory authority(ies) will be granted direct access to the subject's original medical records for verification of clinical trial procedures and/or data without violating the confidentiality of the subject, to the extent permitted by the applicable laws and regulations, and that, by signing a written ICF, the subject is authorizing such access.

Subjects 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. Subjects will be informed whether private information collected from this research will be used for additional research, even if identifiers are removed.

Subjects will be allowed sufficient time to consider participation in this research trial, and have the opportunity to discuss this trial with their family, friends or legally authorized representative, or think about it prior to agreeing to participate.

Informed consent forms will be IRB-approved and subjects will be asked to read and review the ICF. Subjects must sign the ICF prior to starting any study procedures being done specifically for this trial.

Once signed, a copy of the ICF will be given to the subject(s) for their records. The subject(s) may withdraw consent at any time throughout the course of the trial. The rights and welfare of the subject(s) will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

Study personnel may employ recruitment efforts prior to obtaining study consent if a patient-specific screening consent is on record or if the IRB has agreed that chart review is allowed without a fully executed screening consent. In cases where there is not a patient-specific screening consent on record, site clinical staff may pre-screen via chart review and refer potential subjects to the research staff. Research staff would obtain written consent per the standard informed consent process before conducting protocol-specific screening activities.

New information will be communicated by the site PI to subjects who consent to participate in this trial in accordance with IRB requirements. The informed consent document will be updated and subjects will be re-consented per IRB requirements, if necessary. Subjects will be given a copy of all ICFs that they sign.

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

Women of child-bearing potential will not be excluded from the trial. In addition, special populations, e.g., non-English speakers, illiterate or non-writing individuals will not be excluded from this study.

Children and vulnerable populations will be excluded from this study as there is insufficient safety data and this is the first time AVYCAZ combined with ATM will be studied in a clinical trial.

9.4 Subject Confidentiality

Subject confidentiality is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their agents. This confidentiality includes documentation, investigation data, subject's clinical information, and all other information generated during participation in the study. No information concerning the study or the data generated from the study will be released to any unauthorized third party without prior written approval of the DMID and the subject. Subject confidentiality will be maintained when study results are published or discussed in conferences. The study monitor or other authorized representatives of the sponsor or governmental regulatory agencies may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the subjects in this study. The clinical study site will permit access to such records.

All records will be kept locked and all computer entry and networking programs will be carried out with coded numbers only and with password protected systems. All non-clinical specimens, evaluation forms, reports, and other records that leave the site will be identified only by a coded number.

9.5 Certificate of Confidentiality

To protect privacy, a Certificate of Confidentiality will be issued. 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 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 for other research will only occur if consent was obtained from the individual to whom the information or document pertains.

9.6 Costs, Subject Compensation, and Research Related Injuries

There is no cost to subjects for the research tests, procedures, and study product while taking part in this trial. Subjects may be compensated for their participation in this trial. Compensation will be in accordance with the local IRB's policies and procedures, and pursuant to IRB approval.

If it is determined by the site PI that an injury occurred to a subject as a direct result of the tests or treatments that are done for this trial, then referrals to appropriate health care facilities will be provided to the subject. Study personnel will try to reduce, control, and treat any complications from this trial. Immediate medical treatment may be provided by the participating site. No financial compensation will be provided to the subject by the NIAID, NIH to the subject for any injury suffered due to participation in this trial.

10 STATISTICAL CONSIDERATIONS

Detailed methodology for summary and statistical analyses of the data collected in this study will be documented in a Statistical Analysis and PK Plan. The Statistical Analysis and PK Plan document may modify the plans outlined in the protocol; however, any major modifications of the primary endpoints definitions and/or their analyses will also be reflected in a protocol amendment. Additional statistical analyses other than those described in this section may be performed if deemed appropriate and included in the plan.

This is a Phase 1 study of the safety and PK of AVYCAZ with ATM. Each subject will be assigned to a cohort as described in Section 4.3 and have PK samples collected at designated time points. For a subject to be considered "completed" they will have received the full seven days of scheduled study product(s), as well as completing an in person safety follow up.

A subject will be considered to have a complete PK profile if ≥70% of plasma PK samples are collected. If two or more subjects in a dosing cohort have an incomplete PK profile, subjects will be replaced in that dosing cohort to ensure there at least 7 subjects with complete PK profiles in each dosing cohort. For participants who have incomplete PK profile, their available PK data will be considered in the PK analyses.

10.1 Study Hypotheses

The aim of this study is to evaluate the safety and PK of combination doses of AVYCAZ with ATM. As this is a Phase I study, no hypothesis will be tested.

10.2 Sample Size Considerations

This is a Phase I study to investigate the safety and PK of AVYCAZ combined with ATM. The sample size was chosen to obtain reasonable evidence of safety without exposing undue numbers of healthy subjects to combination of AVYCAZ with ATM at this phase of clinical evaluation. Previous experience in Phase I studies has shown that the sample size being proposed is sufficient to fulfil the primary and secondary objectives of the study.

10.3 Treatment Assignment Procedures

This is an open-label study and randomization and masking procedures are not required.

10.4 Final Analysis Plan

10.4.1 Safety

All subjects who receive at least one dose of study product(s) will be included in the safety analysis. AEs [i.e., those beginning after the first dose of study product(s)] will be summarized using frequency counts and percentages by treatment cohort. Graphical presentations may be used, as appropriate. The following summaries will be presented for AEs and SAEs:

- Overall (i.e., regardless of severity or relationship to study product)
- By severity grade (mild, moderate, or severe),
- By relationship to study product,
- By MedDRA level hierarchy (System Organ Class and preferred term).

Unless otherwise specified, at each level of subject summarization in reporting the incidence of the AEs, a subject will be counted once if the subject reported 1 or more events. If more than 1 occurrence of an event is reported, the event of the worst severity or the worst-case relationship assessment will be summarized. Furthermore, listings of deaths (if any), SAEs, and AEs that lead to study discontinuation will be made. The number of volunteers who have any AEs, SAEs, AEs that lead to withdrawal, and AEs judged causally related to study product(s) by the PI will be summarized. Shifts in clinical laboratory data will be tabulated.

For each treatment cohort, the percentage of subjects who experience the following selected dichotomous events will be calculated. Subjects will be counted for each event only once, at the highest observed grade. Event categories include:

- Premature discontinuation of study product(s)
- Any grade 1 or higher laboratory value
- Any grade 1 or higher sign or symptom
- Any grade 1 or higher AEs
- Any grade 1 or higher occurrence of laboratory values, signs, symptoms and/or diagnosis
- Grade 1 or higher of any specific AE that occurs in at least 10% of subjects for any regimen

Separate tables and listings of SAEs will be generated.

Safety laboratory parameters and vital signs will be tabulated for each treatment cohort using descriptive statistics. Laboratory values for hematologic, urinalysis, coagulation, and clinical chemistry panels will be compared to normal ranges as reported by the reference laboratory. Vital signs, physical examinations, clinical laboratory values, and ECG parameters will be

presented by treatment cohort. Continuous variables (hematology [including direct Coombs], clinical chemistry, coagulation, ECGs, urinalysis, and vital signs) will be summarized using descriptive statistics (n, mean, standard deviation, minimum, median, and maximum) by treatment, or scheduled time point, as appropriate. Categorical variables (e.g., urinalysis, etc.) will be summarized in frequency tables (frequency counts and proportion) by treatment, age group, or scheduled time point, as appropriate. Where applicable, data will be summarized for the absolute value at each scheduled assessment, and for the corresponding change from baseline.

10.4.2 Pharmacokinetic Analyses

Pharmacokinetic analyses will be completed by the DCRI Pharmacometrics Center (PMC). 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.

10.4.3 Initial Exploratory Analysis

Concentration-time data will be visualized using box and whisker plots, with investigation of any outliers for erroneous time or concentration data entry. Individual concentration-time plots will be generated on linear and semi-log scales to inform potentially optimal structural model builds for analysis.

10.4.4 Non-Compartmental Pharmacokinetic Analysis

Non-compartmental analysis (NCA) will be used as the initial approach to generated base PK parameter estimates for each individual agent (i.e. CAZ, AVI and ATM). These analyses will be conducted using an appropriate statistical package. This descriptive analysis will allow for comparison to previously published data on each of the individual agents. The following PK parameters will be calculated as appropriate and if possible depending on actual samples collected: maximum plasma concentration (C_{max}) after the first dose on Day 1, maximum plasma concentration at steady state ($C_{ss,max}$), time to C_{max} (t_{max}), time to $C_{ss,max}$ ($t_{ss,max}$), minimum plasma concentration at the end of the dosing interval on Day 1 [C_{min}] and at steady state ($C_{ss,min}$), steady state concentration after CI (C_{ss}), area under the plasma concentration-time curve during the dosing interval on Day 1 [$AUC_{(0\text{-}Tau)}$] and at steady state during the dosing interval [$AUC_{0\text{-}Tau,ss}$], systemic plasma clearance (CL), renal clearance (CL_R), volume of distribution during terminal phase (V_z), dose-normalized exposure parameters after the first and multiple dose, accumulation ratio for C_{max} (RC_{max}) and $AUC_{(0\text{-}Tau)}$ [$RAUC_{(0\text{-}Tau)}$]. Nominal sampling time will be used in PK calculations, unless it is decided that actual times are needed.

10.4.5 Population Pharmacokinetic Analysis

Population pharmacokinetic analysis provides a platform to identify patient covariates which can help explain a portion of the inter-individual variability in selected PK parameters. The non-

linear mixed effects modeling software NONMEM® Version 7.2 (ICON Development Solutions, Ellicott City, MD) will be used to develop the population PK model for CAZ, AVI and ATM concentrations in plasma. Different structural PK models (e.g., 2 and 3 compartment) will be fitted to the concentration-time data of each drug. Between-subject variability on model parameters and different residual error models will be tested. Model development will be guided by goodness of fit plots, plausibility of parameter estimates, and reduction in inter-individual variability for structural and residual error parameters, as well as objective function and shrinkage values.

Upon selection of an appropriate base structural PK model, covariate effects (i.e., age, sex, body size descriptors and renal function) will be evaluated using stepwise forward selection followed by stepwise backward elimination processes. Standard model diagnostic plots and procedures will be used to evaluate model appropriateness. Model validation will be performed using visual predictive check and bootstrapping.

10.4.6 Monte Carlo Simulation

Monte Carlo simulations will be performed using the final population PK model with all statistically significant covariate effects. Simulated exposures of treatment cohorts will be evaluated. Simulated medians and 90% prediction intervals for each treatment cohort will be overlaid on the observed concentration-time data from subjects as a QC check.

10.4.7 Exposure-Response Relationship Analysis

Using the final population PK model, EBE PK parameters and dosing information, simulations will be performed to predict PK exposure of CAZ, AVI and ATM in study subjects. Multiple exposure parameters may be explored. The association between occurrence of ALT or AST elevations and PK exposure of CAZ, AVI and ATM will be explored using standard statistical correlation methodologies.

11 SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA/DOCUMENTS

Each participating site will maintain appropriate medical and research records in compliance with ICH E6, Section 4.9 and regulatory and institutional requirements for the protection of confidentiality of subjects. Each site will permit authorized representatives of the DMID, its designees, and appropriate regulatory agencies to examine (and when required by applicable law, to copy) clinical records for the purposes of QA reviews, audits, and evaluation of the study safety and progress. These representatives will be permitted access to all source data and source documents, which include, but are not limited to, hospital records, clinical and office charts, laboratory notes, memoranda, subjects' memory aid or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, and subject files and records kept at the pharmacy, at the laboratories, and medico-technical departments involved in the clinical trial.

12 QUALITY CONTROL AND QUALITY ASSURANCE

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

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

13 DATA HANDLING AND RECORD KEEPING

13.1 Data Management Responsibilities

The investigator is responsible to ensure the accuracy, completeness, legibility, and timeliness of the data reported. All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data. Black or blue permanent 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.

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

Copies of the eCRF will be provided for use as source data collection forms and maintained for recording data for each subject enrolled in the study. Data reported in the eCRF derived from source data collection forms should be consistent or the discrepancies should be explained.

The sponsor and/or its designee will provide guidance to the site PIs and other study personnel on making corrections to the data collection forms and eCRF.

13.2 Data Coordinating Center/Biostatistician Responsibilities

Data collection is the responsibility of the study personnel at the participating clinical study site under the supervision of the site PI. During the study, the site PI must maintain complete and accurate documentation for the study.

The Emmes Company will serve as the SDDC for this study and will be responsible for data management, quality review, analysis (with the exception of the PK analyses) and reporting of the study data. Adverse events and concomitant medications will be coded using the MedDRA and World Health Organization Drug dictionaries (WHO DD) respectively. The DCRI PMC will be responsible for all analysis of PK data.

13.3 Data Capture Methods

Clinical data (including, but not limited to, AE/SAEs, concomitant medications, medical history, physical assessments, and clinical laboratory values) will be collected on data collection forms by study personnel then entered into eCRFs via a 21 CFR Part 11-compliant internet data entry system provided by the study data coordinating center. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate.

Data will be entered into the eCRF on an ongoing basis and will be reviewed on a continuous basis throughout the study.

13.4 Types of Data

Data for this trial will be collected in a single database. The data will include medical history, demographics, physical examination, vital signs, ECGs, concomitant medications, study product administration, PK collection times, bioanalytical data, PK modelling and safety (e.g., clinical laboratory values and AEs/SAEs). The PK modelling will be completed at the DCRI PMC and a copy transferred to the SDDC database.

13.5 Study Records Retention

Study records and reports including, but not limited to, eCRFs, source documents, ICFs, laboratory test results, and study product disposition records will be retained for 2 years after a marketing application is approved for the study product for the indication for which it is being investigated; or, if no application is to be filed or if the application is not approved for the study product, until 2 years after the investigation is discontinued and the FDA has been notified. 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 the sponsor. It is the responsibility of the sponsor to inform the site PI when these documents no longer need to be retained.

14 CLINICAL MONITORING

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

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

15 PUBLICATION POLICY

Following completion of the study, the lead PI 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 which will register the trial and post results.

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

Refer to:

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

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17 APPENDICES

Appendix A. Schedule of Events

Evaluation	Screening[1]	Visit 1 Enrollment[2]	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9 Discharge	Visit 10 / Final Visit	Unscheduled / Early Termination Visit
	Day -30 to Day -2	Day -1	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 11 (+3)	
Informed consent	X											
Confirmation of eligibility criteria	X	X										
Medical history & Demographics	X											
Height / Weight	X											
Review of changes in medical history		X								X		X
Concomitant medications	X	X	X[3]	X[3]	X[3]	X[3]	X[3]	X[3]	X[3]	X	X	X
Physical examination	X	X								X	X	X
Symptom directed physical examination			X	X	X	X	X	X	X			
Vital signs[4]	X	X	X	X	X	X	X	X	X	X	X	X
ECG[5]	X	X								X	X	X
Assessment of adverse events[6]			X	X	X	X	X	X	X	X	X	X
Assignment to treatment cohort[7]		X										
Study product administration			X	X	X	X	X	X	X			
Clinical chemistry[8]	X	X		X		X		X		X	X	X
LFTs[9]							X		X			
Hematology[10]	X	X		X		X		X		X	X	X
Coagulation test[11]	X	X		X		X		X		X	X	X
Viral serology[12]	X											
Coombs test	X									X		X
Urinalysis[13]	X	X				X				X	X	X

Evaluation	Screening[1]	Visit 1 Enrollment[2]	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9 Discharge	Visit 10 / Final Visit	Unscheduled / Early Termination Visit
	Day -30 to Day -2	Day -1	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 11 (+3)	
Serum hCG pregnancy test[14]	X	X										
Alcohol Screen breathalyzer	X	X										
Urine cotinine test	X	X										
Urine drug screen[15]	X	X										
Plasma PK[16]			X		X		X		X	X		X[17]
Urine PK[18]			X					X				

1. Screening evaluations must be completed within 30 days prior to enrollment
2. All baseline procedures should be done before study intervention
3. Review for any new medication only
4. Vital Signs: collected after 10 minutes of resting in supine position and include systolic and diastolic blood pressures (mmHg), pulse rate (BPM), respiratory rate, and body temperature (oral measurement). Day 1 - prior to dosing on Day 1 and 1 hour after starting AM dose. Days 2-8 will be obtained each morning
5. 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)
6. Monitoring for adverse events will begin after the first dose of study product
7. Assignment to dosing cohort: Must occur after all inclusion and exclusion criteria have been confirmed
8. Clinical Chemistry: sodium, potassium, chloride, bicarbonate, BUN, creatinine, glucose, albumin, total protein, total bilirubin, ALT, AST, ALP and LDH
9. Liver function tests (LFTs): ALT, AST, ALP, and total bilirubin
10. Hematology: Hemoglobin, hematocrit, WBC, WBC differential, RBC, platelets
11. Coagulation: PT and PTT
12. Viral serology: HIV, HBsAg, HCV antibody
13. Urinalysis: Dipstick urinalysis, including protein, glucose, ketones, bilirubin, blood, nitrites, LCE, urobilinogen, specific gravity, and pH
14. Serum pregnancy test on Day-1 must be completed within 48 hours prior dosing. Results must be obtained prior to study product(s) administration
15. Urine drug screen for: amphetamines, barbiturates, benzodiazepines, cocaine metabolites, opiates, cannabinoids and phencyclidine
16. Plasma PK collection times: See Section 7.2.2.1
17. Unscheduled visit – plasma PK collection is optional
18. Urine PK collection times: See Section 7.2.2.2

Appendix B. Toxicity Table

Clinical Adverse Events

CARDIOVASCULAR TOXICITY	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Arrhythmia		Asymptomatic; transient signs; no medical intervention required	Recurrent/persistent; symptomatic medical intervention required
Hemorrhage, Blood Loss	Estimated blood loss <100 mL	Estimated blood loss ≥100 mL, no transfusion required	Transfusion required
QTc (Fridericia's correction)[1] or QTcb (Bazett's)[1]	Asymptomatic, QTc interval 450-479 msec OR Increase in interval 20-30 msec above baseline	Asymptomatic, QTc interval 480-499 msec OR Increase in interval 31-50 msec above baseline	Asymptomatic, QTc interval ≥ 500 msec OR Increase in interval ≥ 51 msec above baseline
PR Interval (prolonged)[1]	PR interval 0.21-0.25 sec	PR interval >0.25	Type II 2nd degree AV block OR Ventricular pause >3.0 sec
RESPIRATORY TOXICITY	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**
Cough	Transient; no treatment	Persistent cough	Interferes with daily activities
Bronchospasm, Acute	Transient; no treatment; 71% - 80% FEV1 of predicted peak flow	Requires medical intervention; 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 daily and usual social activity OR requires treatment
GASTROINTESTINAL TOXICITY	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**
Nausea	No interference with activity	Some interference with activity	Prevents daily activities
Vomiting	No interference with activity OR 1 - 2 episodes/24 hours	Some interference with activity OR >2 episodes/24 hours	Prevents daily activity OR requires IV hydration OR requires medical intervention
Diarrhea	2 - 3 loose or watery stools or <400 g24 hours	4 - 5 loose or watery stools or 400 - 800 g/24 hours	6 or more loose or watery stools or >800g24 hours OR requires IV hydration OR requires medical intervention

[1] Inclusion dependent upon protocol requirements.

LOCAL REACTIONS	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Pain	Does not interfere with activity	Repeated use of non-narcotic pain reliever >24 hours OR interferes with activity	Any use of narcotic pain reliever OR prevents daily activity
Tenderness	Discomfort only to touch	Discomfort with movement	Significant discomfort at rest
Erythema/Redness[2]	2.5 - 5 cm	5.1 - 10 cm	>10 cm
Induration/Swelling[3]	2.5 - 5 cm and does not interfere with activity	5.1 - 10 cm OR interferes with activity	>10 cm OR prevents daily activity
SYSTEMIC REACTIONS	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**
Allergic Reaction	Pruritus without rash	Localized urticaria OR requires oral therapy	Generalized urticaria; angioedema OR anaphylaxis OR requires epinephrine
Headache	No interference with activity	Repeated use of non-narcotic pain reliever >24 hours OR some interference with activity	Significant; any use of narcotic pain reliever OR prevents daily activity OR requires triptans
Fatigue	No interference with activity	Some interference with activity	Significant; prevents daily activity
Myalgia	No interference with activity	Some interference with activity	Significant; prevents daily activity
All Other conditions	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**
Illness or clinical adverse event (as defined according to applicable regulations)	No interference with activity	Some interference with activity not requiring medical intervention	Prevents daily activity and requires medical intervention

[2] In addition to grading the measured local reaction at the greatest single diameter, the measurement should be recorded as a continuous variable.
[3] Induration/Swelling should be evaluated and graded using the functional scale as well as the actual measurement.

Laboratory and Vital Signs Reference Ranges, Eligibility Ranges, and Toxicity Grading

Blood, Serum, or Plasma Chemistries[1]	Reference Range[2]	Eligibility Range[3]	LO/HI/N[4]	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Sodium (mEq/L)	135 - 145	135 - 145	LO	132 - <LLN	130 - 131	<130
			HI	>ULN - 148	149 - 150	>150
Potassium (mEq/L)	3.5 – 5.0	3.5 – 5.0	HI	>ULN - 5.2	5.3 - 5.4	>5.4
			LO	>LLN - 3.1	<3.1 - 3.0	<3.0
Blood Urea Nitrogen (BUN, mg/dL)	7 - 20	7 - 20	HI	21 - 26	27 - 31	>31
Creatinine (mg/dL)	0.5 - 1.2	0.5 – 1.2	HI	>ULN - 1.7	1.8 - 2.0	>2.0
Glucose (mg/dL)	<70	<70	LO	65 - 69	55 - 64	<55
	70 - 99	70 - 99	HI[5]	>ULN - 120	121 - 130	>130
	70 - 140	70 - 140	HI[6]	141 - 159	160 - 200	>200
Total Protein (g/dL)	5.8 - 7.8	5.8 - 7.8	LO	5.2 - <LLN	4.8 - 5.1	<4.8
Bilirubin, serum total (mg/dL)	0.4 - 1.5	0.4 - 1.5	HI	1.6 - 2.0	2.1 - 2.5	>2.5
ALT (U/L)	Female: 14-54	Female: 14-54	HI	>ULN - 105	106 - 175	>175
	Male: 17-63	Male: 17-63				
AST (U/L)	15 - 41	15 - 41	HI	42 - 105	106 - 175	>175
Alkaline phosphatase (U/L)	24 - 110	24 - 110	HI	111 - 240	241 - 360	>360

[1] Depending upon the lab used, references ranges, eligibility ranges and grading may be split out by sex and/or age.
[2] Reference range of site laboratory
[3] Laboratory values acceptable for eligibility for enrollment
[4] High, Low, Not Graded
[5] Fasting
[6] Non-fasting

Hematology	Reference Range[7]	Eligibility Range[8]	LO/HI/N[9]	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Hemoglobin (Females) (g/dL)	12.0-15.5	12.0-15.5	LO	11.0 - 11.9	9.5 - 10.9	<9.5
Hemoglobin (Males) (g/dL)	13.7-17.3	13.7-17.3	LO	12.0 – 13.6	10.0 - 11.9	<10.0
White Blood Cell Count (WBC, K/CUMM)	3.2-9.8	3.2-9.8	HI	9.9 – 14.99	15.00 - 20.00	>20.00
			LO	2.50 - 3.19	1.50 - 2.49	<1.50
Lymphocytes (K/CUMM)	0.6-4.2	0.6-4.2	LO	0.50 – 0.59	0.40 - 0.49	<0.4
Neutrophils (K/CUMM)	2.0-8.6	2.0-8.6	LO	1.50 – 1.99	1.00 - 1.49	<1.00
Eosinophils (K/CUMM)	0.0-0.7	0.0-0.7	HI	>ULN - 0.74	0.75 - 1.50	>1.50
Platelets (K/CUMM)	150-450	150-450	LO	120 - 149	100 - 119	<100
Coagulation						
Prothrombin time (PT, seconds)	9.5-13.1	9.5-13.1	HI	>ULN - 14.4	14.5 - 15.7	>15.7
Partial thromboplastin time (PTT or aPTT, seconds)	26.8-37.1	26.8-37.1	HI	>ULN - 42.1	42.2 - 50.0	>50.0
Urine*						
Protein (dipstick)	0	0	HI	1+	2+	>2+
Glucose (dipstick)	0	0	HI	1+	2+	>2+

Vital Signs	Mild (Grade 1)[10]	Moderate (Grade 2)	Severe (Grade 3)
Fever (°F)	100.4 - 101.1	101.2 - 102.0	≥102.1

[7] Reference range of site laboratory
[8] Laboratory values acceptable for eligibility for enrollment
[9] High, Low, Not Graded
[10] If initial bound of Grade 1 has gap from reference range or eligibility range, calculations based on NEJM reference ranges

Vital Signs	Mild (Grade 1)[10]	Moderate (Grade 2)	Severe (Grade 3)
Tachycardia - beats per minute[11]	101 - 115	116 - 130	>130 or ventricular dysrhythmias
Bradycardia - beats per minute[12]	50 – 54 or 45 – 49 if baseline 50-59 bpm	45 – 49 or 40 - 44 if baseline 50-59 bpm	< 45 or <40 if baseline 50-59 bpm
Hypertension (systolic) - mm Hg[13]	141 - 150	151 - 160	>160
Hypertension (diastolic) - mm Hg	91 - 95	96 - 100	>100
Hypotension (systolic) - mm Hg	85 - 89	80 - 84	<80
Tachypnea - breaths per minute	23 - 25	26 - 30	>30

[11] Expanded heart rate limits can be considered with normal ECGs.
[12] Expanded heart rate limits can be considered with normal ECGs.
[13] Assuming subject is awake, resting, and supine; for AE, 3 measurements on the same arm with concordant results.