CLINICAL RESEARCH IN INFECTIOUS DISEASES

SAFETY STATISTICAL ANALYSIS PLAN
for
DMID Protocol: 16-0011
Study Title:
A Phase IV Open-Label Pharmacokinetic Study of Minocycline for Injection Following a Single Infusion in Critically-Ill Adults (ACUMIN)

NCT03369951

Version 1.0

DATE: 11JUL2019

STUDY TITLE

Protocol Number Code:	**DMID Protocol: 16-0011**
Development Phase:	Phase IV
Products:	Minocin® IV (minocycline hydrochloride injection)
Form/Route:	Intravenous infusion
Indication Studied:	Infection with Gram-negative bacteria
Sponsor:	Division of Microbiology and Infectious Diseases National Institute of Allergy and Infectious Diseases National Institutes of Health
Clinical Trial Initiation Date:	08FEB2018
Clinical Trial Completion Date:	TBD
Date of the Analysis Plan:	11JUL2019
Version Number:	1.0

This study was performed in compliance with Good Clinical Practice.

TABLE OF CONTENTS

Table of Contents *(continued)*

Table of Contents *(continued)*

LIST OF ABBREVIATIONS

AE	Adverse Event
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
AUC	Area under plasma drug concentration versus time curve ratio
$AUC_{0\text{-}24}$	Area under the plasma drug concentration-time curve from 0 to 24 hours after a dose
$AUC_{0\text{-}last}$	Area under the plasma drug concentration-time curve from 0 to the time of the last quantifiable sample after a dose
$AUC_{0\text{-}\infty}$	Area under the plasma drug concentration-time curve from 0 to infinity after a dose
C	Celsius
CI	Confidence Interval
CL	Clearance
CLd	Distribution clearance
C_{max}	Maximum plasma drug concentration
C_{24}	Plasma drug concentration at 24-hours after a dose
CrCl	Creatinine Clearance
CRF	Case Report Form
DMID	Division of Microbiology and Infectious Diseases
DSMB	Data and Safety Monitoring Board
EDC	Electronic Data Capture
ER	Emergency Room
F	Fahrenheit
*f*AUC:MIC	Free-drug AUC:MIC ratio
H	Hour
ICH	International Conference on Harmonisation
ICU	Intensive Care Unit
IRB	Institutional Review Board
IV	Intravenous
L	Liter
MedDRA	Medical Dictionary for Regulatory Activities

List of Abbreviations *(continued)*

mEq	Milliequivalent
mg	Milligram
mL	Milliliter
MOP	Manual of Procedures
N	Number (typically refers to subjects)
NIH	National Institutes of Health
PD	Pharmacodynamics
PI	Principal Investigator
PK	Pharmacokinetics
QA	Quality Assurance
QC	Quality Control
RBC	Red Blood Cell
RRT	Renal Replacement Therapy
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SD	Standard Deviation
SDCC	Statistical and Data Coordinating Center
SMC	Safety Monitoring Committee
SOC	System Organ Class
SOP	Standard Operating Procedures
SUSAR	Suspected Unexpected Serious Adverse Reaction
U	Units
Vc	Volume of distribution in central compartment
Vp	Volume of distribution in the peripheral compartment
Vss	Volume of distribution at steady state
WBC	White Blood Cell
WHO	World Health Organization

1. PREFACE

The Safety Statistical Analysis Plan (SAP) for "A Phase IV Open-Label Pharmacokinetic Study of Minocycline for Injection Following a Single Infusion in Critically-Ill Adults (ACUMIN)" (DMID Protocol 16-0011) describes and expands upon the statistical information presented in the protocol. The main text of this Safety SAP discusses analysis of safety endpoints for the study. Analysis of all Pharmacokinetic (PK) endpoints is presented in the PK SAP.

This document describes all planned analyses and provides reasons and justifications for these analyses. It also includes sample tables, listings, and figures planned for the final analyses. Regarding the final analyses and Clinical Study Report (CSR), this SAP follows the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) Guidelines, as indicated in Topic E3 (Structure and Content of Clinical Study Reports), and more generally is consistent with Topic E8 (General Considerations for Clinical Trials) and Topic E9 (Statistical Principles for Clinical Trials). The structure and content of the SAP provides sufficient detail to meet the requirements identified by the FDA and ICH, while all work planned and reported for this SAP will follow internationally accepted guidelines published by the American Statistical Association and the Royal Statistical Society for statistical practice.

Any deviation from this SAP will be described and justified in protocol amendments and/or in the CSR, as appropriate. The reader of this SAP is encouraged to also review the study protocol for details on conduct of the study and the operational aspects of clinical assessments.

2. INTRODUCTION

This is a Phase IV, multi-center, open-label pharmacokinetic trial studying the pharmacokinetics and pharmacodynamics of a single dose of Minocin IV in 50 evaluable, ICU patients already receiving antimicrobial therapy for a known or suspected Gram-negative infection. Up to 67 patients will be enrolled to obtain 50 evaluable subjects. Subjects who received any study product (started infusion), regardless of evaluable status, will be included in the safety population used for all safety analyses.

Each subject receives a single 200 mg dose of Minocin IV infused over approximately 60 minutes. Each subject has up to 7 PK samples collected (1 pre-dose, 6 post-dose) at designated time points over a ~48-hour period following the start of the Minocin IV infusion.

2.1. Purpose of the Analyses

The main text of this Safety SAP discusses analysis of the safety endpoints collected to assess the safety of a single 200 mg dose of Minocin IV infused over approximately 60 minutes. This Safety SAP includes only an overview of the PK population exclusions. Analysis of all PK objectives and associated endpoints are described in the PK SAP.

3. STUDY OBJECTIVES AND ENDPOINTS

3.1. Study Objectives

3.1.1. Safety Objectives

Exploratory objectives:

- To evaluate changes of physiological parameters following a single infusion of minocycline among critically-ill adults.

3.1.2. PK Objectives

Primary objectives:

- To characterize minocycline PK at the population level in critically-ill adults, with illness known or suspected to be caused by infection with Gram-negative bacteria.
- To assess patient-level and clinical covariates associated with minocycline pharmacokinetic properties in critically-ill adults, with illness known or suspected to be caused by infection with Gram-negative bacteria.

Exploratory objectives:

- To predict the distribution of concentration-time profiles observed with the FDA approved minocycline dosing regimen in critically-ill adults, with illness known or suspected to be caused by infection with Gram-negative bacteria via simulations.
- To assess whether dosing adjustments for the approved FDA minocycline dosing regimen are needed based on identifiable patient-level and clinical covariates among critically-ill adults, with illness known or suspected to be caused by infection with Gram-negative bacteria via simulations.
- Conduct simulation studies to determine the ability of the FDA approved minocycline dosing scheme to achieve critical pharmacokinetic-pharmacodynamic targets against the range of minocycline MIC values observed with infections due to Acinetobacter baumannii, including MDR strains.

3.2. Endpoints

3.2.1. Safety Endpoints

3.2.1.1. Exploratory Outcome Measures

- Change from baseline at 48hr post-dose in:
  - liver function tests
  - magnesium
  - serum creatinine

3.3. Study Definitions and Derived Variables

Serious Adverse Events

Only Serious Adverse Events (SAEs) are captured in this protocol. An SAE is defined as an adverse event (AE) meeting one of the following conditions:

- Results in death during the period of protocol defined surveillance.
- Is life-threatening (defined as a subject at immediate risk of death at the time of the event).
- Requires inpatient hospitalization or prolongation of existing hospitalization during the period of protocol defined surveillance.
- Results in congenital anomaly or birth defect.
- Results in a persistent or significant disability/incapacity.
- Any other important medical event that may not result in death, be life threatening, or require hospitalization, may be considered an SAE when, based upon appropriate medical judgment, the event may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above. 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 are assessed by the clinician, the following guidelines will be used to quantify intensity:

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

The clinician's assessment of an SAE's relationship to study drug is part of the documentation process, but it is not a factor in determining what is or is not reported in this study. If there is any doubt as to whether a clinical observation is an SAE, the event should be reported. The relationship to study product must be assessed for SAEs using the terms: related or not related. In a clinical trial, the study product must always be suspect. To help assess, the following guidelines are used:

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

Creatine Clearance (CrCl)

CrCl will be calculated using the Cockcroft-Gault formula:

CrCl = [(140-age(years)) x (Wt(kg)) x (0.85 if female)] / (72 x Serum Creatinine (mg/dL))

For additional study definitions and derived variables related to PK endpoints, refer to the PK SAP.

4. INVESTIGATIONAL PLAN

4.1. Overall Study Design and Plan

This is a Phase IV, multi-center open-label pharmacokinetic trial studying the pharmacokinetics and pharmacodynamics of a single dose of Minocin IV in 50 evaluable, ICU patients already receiving antimicrobial therapy for a known or suspected Gram-negative infection. Once patients are confirmed as eligible, and informed consent is obtained, patients will be enrolled into the study.

Each subject will receive a single 200 mg dose of Minocin IV infused over approximately 60 minutes. Each subject will have up to 7 PK samples collected (1 pre-dose, 6 post-dose) at designated time points over a ~48-hour period following the start of the Minocin IV infusion. A diagram of the overall study design is shown in Figure 1. Enrollment is estimated to be completed in approximately 16 months. There will not be any stratification. Subjects will be expected to be in the trial for approximately 2 days after they are enrolled. Additional follow-up may be required.

Up to 67 subjects will be enrolled in order to obtain 50 PK evaluable subjects in the study. To be considered PK evaluable, a subject must (1) have the baseline pre-dose PK sample collected (2) receive the full infusion of study drug, (3) have at least 3 PK samples collected in the first 12 hours post dose, (4) have at least 1 PK sample collected 24-48 hours post dose, and (5) PK specimens processed per the Manual of Procedures (MOP). All subjects who received any study product (started infusion), regardless of PK evaluable status, will be included in the safety population used for all safety analyses.

Screening will take place within 48 hours of enrollment and subject satisfying all of the inclusion criteria and none of the exclusion criteria will be enrolled. At the baseline visit, subjects will receive the single 200 mg dose of Minocin IV infused over approximately 60 minutes. The start of study infusion will be at the 0-hour (h) time point. Prior to study product administration the following procedures will be completed: collection of concomitant medications, blood chemistry, liver function tests, hematology, vital signs, weight, PK blood samples, serum creatinine and magnesium.

Safety assessments collected post dose will include:

- Vital signs collected at 1 h, 24 h and 48 h post start of study drug infusion
- Serum creatinine collected at the following time points: 1 h, 4 h, 12 h, 24 h, 36 h and 48 h post start of study drug infusion
- Magnesium collected at 24 h and 48 h post start of study drug infusion
- Liver test parameters collected at 48 h post start of study drug infusion
- Hematology collected at 24 h and 48 h post start of study drug infusion
- SAEs will be collected from the start of the investigational drug infusion and will continue for 24 h from that point.

Additional covariate information collected post dose will include:

- Receipt of any renal replacement therapies (RRT) will be collected from 1 h post start of infusion through 48 h post start of infusion.
- Collection of concomitant medications, including IV medications administered (collected from 24 hours prior to the start of infusion through 48 hours post start of infusion (total of 72 hours).

The final study visit will be at 48-hour post start of infusion time point. SAE follow up visits to follow up on any SAEs occurring up to 24 hours post start of drug infusion may be added as needed.

4.2. Discussion of Study Design, Including the Choice of Control Groups

As the main focus of this study is to study the pharmacokinetics and pharmacodynamics of a single dose of Minocin IV for patients in the ICU, there is no control group and all subjects receive the same dose of Minocin IV with the same PK blood collection time points.

4.3. Selection of Study Population

The study population for this trial is 50 critically-ill adult males and females in the ICU with illness known or suspected to be caused by infection with Gram-negative bacteria. Up to 67 subjects will be enrolled to obtain 50 evaluable subjects. This population was selected since no previous research has looked into the minocycline PK indices among ICU patients . PK studies of other antibiotics in critically ill patients have shown discrepancies between the anticipated PK parameters based on healthy volunteers or less ill patients. These differences included altered volumes of distribution, enhanced renal clearance, and altered drug metabolism, ultimately resulting in altered blood concentration time profiles. This study will therefore develop a population PK model to describe the PK profile of minocycline in patients in the ICU.

Eligibility criteria from version 3.0 of the protocol are listed below:

4.3.1. Subject Inclusion Criteria

Subjects must meet all of the following criteria in order to be eligible for the study:

1. Male or female ≥18 years of age
2. Subject is in the ICU, or is being admitted to the ICU
3. Known or suspected Gram-negative infection for which the subject is receiving systemic antibiotics, and which was the reason for admission to the ICU, or reason for persistent need for ICU care
4. Expectation, in the judgment of the investigator, that the subject will remain admitted in the hospital for at least 48 hours following enrollment and that all study procedures will be completed
5. Expectation that intravenous access will be sufficient for drug infusion and either intravenous or arterial access will be sufficient to allow for all protocol required blood sampling to occur
6. The subject, or legally authorized representative (LAR), is able and willing to provide signed informed consent

4.3.2. Subject Exclusion Criteria

Subjects who meet any of the following criteria at baseline will not be enrolled in the study:

1. History of significant hypersensitivity or allergic reaction to tetracycline antibiotics
2. Receipt of oral or intravenous tetracycline class drugs within 7 days of enrollment (e.g., minocycline, tetracycline, tigecycline, doxycycline)
3. Use of isotretinoin within 2 weeks of enrollment into the study
4. Major surgery[1] within 48 hours prior to enrollment

[1]*Major surgery is defined as "the opening of either a body cavity or the mesenchymal barrier, using general anesthesia"*

5. Pregnant or breastfeeding women
6. Patient is being treated for intracranial hypertension
7. Any condition that, in the judgment of the investigator, precludes participation because it could affect subject safety[2]

   [2]*Subjects on, or who may be considered for Renal Replacement Therapy (RRT) during the study period are not excluded from participating in the study.*
8. Receipt of an investigational study product within 7 days prior to enrollment. Investigator discretion should be used when longer acting agents have been used in the previous 30 days

4.4. Treatments

4.4.1. Treatments Administered

Minocin® IV (minocycline hydrochloride injection) 200mg administered intravenously as a single infusion over approximately 60 minutes.

4.4.2. Identity of Investigational Product(s)

Minocin IV is supplied as a sterile yellow to amber lyophilized powder for intravenous infusion in a single-use 10 mL glass vial with a rubber stopper and aluminum overseal. Each vial contains108 mg of minocycline hydrochloride (equivalent to 100 mg of minocycline), 269 mg magnesium sulfate heptahydrate (2.2 mEq of magnesium) (an inactive ingredient) and sodium hydroxide (to adjust pH). When reconstituted with 5 mL of Sterile Water for Injection USP the pH ranges from 4.5 to 6.0. Further information can be found in the MOP and Minocin® (minocycline) for Injection Package Insert.

4.4.3. Method of Assigning Subjects to Treatment Groups (Randomization)

This is a single arm study. There are no randomization procedures. Subjects will be enrolled online through the enrollment module of AdvantagEDC℠, Emmes' Internet Data Entry System (IDES).

4.4.4. Selection of Doses in the Study

200mg of Minocin® IV will be administered intravenously as a single infusion over approximately 60 minutes. While there is longstanding clinical use experience with minocycline in patients, PK studies of minocycline are relatively limited and were conducted in the 1970s in healthy volunteers. Pharmacokinetic data from a single study in 10 healthy male subjects is reported in the USPI and states that following a single dose of Minocin IV 200 mg, serum concentrations of minocycline ranged from 2.52 µg/mL to 6.63 µg/mL (average of 4.18 µg/mL) at the end of infusion and 0.82 µg/mL to 2.64 µg/mL (average of 1.38 mcg/mL) after 12 hours. The label further states that following administration of Minocin IV 100 mg administered every 12 hours for three days to five healthy male subjects, serum concentrations of minocycline ranged from 1.4 µg/mL to 1.8 µg/mL at the end of the dosing interval [1].

4.4.5. Selection and Timing of Dose for Each Subject

All enrolled subject will receive 200 mg of Minocin IV administered intravenously as a single infusion over approximately 60 minutes.

4.4.6. Blinding

This is an unblinded study. Masking is not needed.

4.4.7. Prior and Concomitant Therapy

Concomitant and treatment medications will be collected from 24 hours prior to the start of infusion through 48 hours post start of infusion (total of 72 hours), as well as the SAE follow-up visit as needed.

Receipt of any RRTs will be collected from 1-hour post start of infusion through 48 hours post start of infusion.

4.4.8. Treatment Compliance

Because the study drug will be administered at the clinic by site personal, subject compliance is not expected to be an issue. Study drug compliance including start and stop time of infusion, volume administered, and the occurrence of any infusion interruptions (greater than 10 minutes) will be document by site staff.

4.5. Safety Variables

The following section describes the variables related to the safety endpoints, safety related variables collected throughout the study.

For a detailed schedule of procedures refer to Table 1.

In this study, only SAEs will be collected and summarized. The type, incidence and severity of treatment-emergent SAEs will be recorded from the start of administration of Minocin IV at 0 h to 24 h after the start of infusion. Refer to section 3.3 for definitions of SAE, severity and relatedness.

The following safety laboratory parameters and vital signs will be summarized for each time point collected. Change from baseline will be summarized for all post dose time points. Laboratory parameters and vital signs will be summarized as continuous variables and will not be graded for severity.

- Serum creatinine collected at baseline and 1 h (+0.5h), 4 h (+1 h), 12 h (±2 h), 24 h (±2 h), 36 h (±2 h), and 48 h (±2 h) post start of infusion.
- Magnesium collected at baseline, 24 h (±2 h) post start of infusion and 48 h (±2 h) post start of infusion
- Liver Function Tests (AST, ALT, alkaline phosphatase, albumin, total bilirubin) collected at baseline and 48 h (±2 h) post start of infusion.
- Hematology laboratory parameters (hemoglobin, hematocrit, white blood cell count (WBC), WBC differential, red blood cell count, platelets) collected at baseline, 24 h (±2 h) post start of infusion and 48 h (±2 h) post start of infusion.
- Vital Signs (systolic blood pressure, diastolic blood pressure, heart rate, respiratory rate, and temperature) collected at baseline and 1 h (+0.5h), 24 h (±2 h), and 48 h (±2 h) post start of infusion.

The baseline value used for calculating change from baseline for the above safety laboratory parameters and vital signs will be defined as the last value obtained prior to the start of infusion of study product. The following time frames will be used for the baseline measurement for collection of these variables:

- Serum creatinine collected just prior to the start of study drug infusion.
- Magnesium collected just prior to the start of study drug infusion.

- Liver function tests (Aspartate Aminotransferase (AST), Aspartate Aminotransferase (ALT), Alkaline Phosphatase, Albumin, Total Bilirubin) collected within 8 hours prior to the start of study drug infusion.
- Blood chemistry collected within 8 hours prior to the start of study drug infusion.
- Hematology collected within 8 hours prior to the start of study drug infusion.
- Vital signs, collected as close to the start of the study drug infusion as possible.

The following additional laboratory parameters are collected at baseline or screening only and will be summarized only for the pretreatment timepoint:

- Chemistry laboratory parameters (Potassium, Sodium, Chloride, Bicarbonate, Glucose, Blood Urea Nitrogen) collected at baseline.
- Urinalysis laboratory parameters (Protein, Glucose, Ketones, Bilirubin, Blood, Nitrites, LCE, Urobilinogen, Specific Gravity, and pH) collected at screening.

5. SAMPLE SIZE CONSIDERATIONS

This is a Phase IV, multi-center open-label pharmacokinetic trial studying the pharmacokinetics and pharmacodynamics of a single dose of Minocin IV in 50 evaluable, ICU patients already receiving antimicrobial therapy for a known or suspected Gram-negative infection. The sample size for this study was selected in accordance with current guidance for PK studies [2].

Up to 67 subjects will be enrolled in order to obtain 50 PK evaluable subjects in the study. Subjects who are not evaluable for the PK analysis will be replaced.

6. GENERAL STATISTICAL CONSIDERATIONS

6.1. General Principles

All continuous variables will be summarized using the following descriptive statistics: N (non-missing sample size), mean, standard deviation (SD), median, maximum (max) and minimum (min). The frequency and percentages (based on the non-missing sample size) of observed levels will be reported for all categorical measures. When 95% CIs are given for a percent, exact (Clopper-Pearson) CIs will be used.

In general, all data will be listed, sorted by site and subject, and when appropriate by time period within subject. All summary tables will be annotated with the total population size relevant to that table, including any missing observations.

6.2. Timing of Analyses

The final analysis will be performed after database lock. There are no planned interim analyses.

6.3. Analysis Populations

6.3.1. Safety Population

All subjects who began infusion of study product will be included in the Safety Population.

6.3.2. Pharmacokinetics Population

To be considered PK evaluable, a subject must have the baseline pre-dose PK sample collected, receive the full infusion of study drug, have at least 3 PK samples collected in the first 12 hours post dose, have at least 1 PK sample collected 24-48 hours post dose, and PK specimens must be processed per the MOP.

For additional details regarding the Pharmacokinetics Population refer to the PK SAP.

6.4. Covariates and Subgroups

The protocol does not define any formal subgroup analyses.

6.5. Missing Data

All attempts will be made to collect all data per protocol. No imputation will be performed for missing safety values and outliers will not be excluded from the safety analysis. The amount of missing data and reasons for missingness if available will be summarized in the body of the analysis report.

6.6. Interim Analyses and Data Monitoring

No interim analyses are planned. A safety summary report will be generated for review by the Safety Monitoring Committee (SMC) if the halting rule (see Protocol Section 9.5) is met. An aggregate review of SAEs will be presented annually to the SMC.

6.7. Multicenter Studies

Data will be pooled across all clinical sites. Center effects are not anticipated because the sites are using standardized procedures for study product administration, assessment of unsolicited adverse events, and collection of vital signs and laboratory parameters.

6.8. Multiple Comparisons/Multiplicity

The safety data summarized in this report will not be adjusted for multiple comparisons.

7. STUDY SUBJECTS

7.1. Disposition of Subjects

The disposition of subjects in the study will be tabulated by site (Table 3). The table shows the total number of subjects screened, enrolled, received any study product, completed infusion of study product, completed all PK blood draws, the number completing the study and the number of subjects determined to be PK evaluable.

A flowchart showing the disposition of study subjects will be included (Figure 2). This figure will present the number of subjects screened, enrolled, lost to follow-up, and analyzed. A listing of subjects who discontinued dosing or terminated from study follow-up and the reason will be included in Listing 1.

The composition of the Safety and PK analysis populations, including reasons for subject exclusion is presented in Table 4. Subjects who were excluded from any analysis population will be listed along with reason for exclusion (Listing 4).

Table 5 will present a summary of the reasons that subjects were screened but not enrolled.

7.2. Protocol Deviations

A summary of subject-specific protocol deviations will be presented by the reason for the deviation and the deviation category for all subjects (Table 2). This table will provide both the number of subjects and the number of deviations for each category. Deviations that are considered major deviations that will be reviewed for possible subject exclusion from the PK population include: incomplete infusion of study product, missing PK blood draws at PK time points, and PK samples not processed per the MOP. All subject-specific protocol deviations and non-subject specific protocol deviations will be included in Appendix 3 as data listings (Listing 2 and Listing 3, respectively).

8. EFFICACY EVALUATION

There are no efficacy endpoints for this protocol.

9. SAFETY EVALUATION

All safety analyses will be presented using the safety population.

When calculating the incidence of SAEs (i.e., on a per subject basis), each subject will be counted once and any repetitions of unsolicited SAEs within a subject (by treatment) will be ignored for events coded in the same category by the Medical Dictionary for Regulatory Activities (MedDRA®). The denominators for percent values will be indicated within the table or table header and denominators will consist of the maximal size of the safety population in the indicated observation period.

9.1. Demographic and Other Baseline Characteristics

All safety analyses will be presented using the safety population.

Summaries of sex, ethnicity, race, age, weight, height, BMI, and baseline CrCl will be presented by site (Table 6 and Table 7). Ethnicity is categorized as Hispanic or Latino, or not Hispanic and not Latino. In accordance with NIH reporting policy, subjects may self-designate as belonging to more than one race or may refuse to identify a race, the latter reflected in the CRF as "No" to each racial option.

Individual subject listings (Appendix 3) will be presented for all demographics (Listing 5).

9.1.1. Prior and Concurrent Medical Conditions

All current illnesses and past pre-existing medical conditions will be coded using MedDRA dictionary version 20.1 or higher. Summaries of subjects' pre-existing medical conditions by MedDRA system organ class (SOC) will be presented in Table 8. Individual subject listings will be presented for all medical conditions (Listing 6).

9.2. Measurements of Treatment Compliance

Any subjects who were enrolled but did not begin infusion of study drug will be presented by site as part of the subject disposition table (Table 3). Subject who did not receive the full infusion of study drug will be listed (Listing 7).

9.3. Adverse Events

Only unsolicited SAEs will be captured in this protocol. When calculating the incidence of serious adverse events (i.e., on a per subject basis), each subject will only be counted once and any repetitions of adverse events within a subject will be ignored; the denominator will be the number of subjects in the safety population. All serious adverse events reported will be included in the summaries and analyses.

9.3.1. Solicited Events and Symptoms

No solicited adverse event will be collected.

9.3.2. Unsolicited Serious Adverse Events

The proportion of subjects reporting at least one unsolicited serious adverse event will be summarized by MedDRA system organ class and preferred term. Denominators for percentages are the number of subjects in the Safety Population.

A brief overall summary of SAEs will be shown in Table 9.

The following summaries for unsolicited serious adverse events will be presented by MedDRA system organ class and preferred term:

- Frequency (number and percent of subjects with an SAE of mild severity or greater, with 95% CIs) and number of events, regardless of severity or relationship to study product (Table 10).
- Frequency of SAEs by severity and relationship to study product (Table 11);
- Bar chart displaying the frequency (number of subjects) of serious related adverse events by severity and MedDRA system organ class (Figure 3);
- Bar chart displaying the incidence (number of occurrences) of serious related adverse events by maximum severity and MedDRA system organ class (Figure 4);

9.4. Deaths, Serious Adverse Events and other Significant Adverse Events

A listing of serious adverse events by subject will be presented in Table 12. The listing will include Subject ID, Adverse Event Description, Adverse Event Onset Date/End Date, Days Post Dose, Reason Reported as an SAE, Relationship to Treatment, Alternate Etiology if not Related, action taken with study treatment, whether subject discontinued due to AE, outcome, whether the event was a suspected unexpected serious adverse reactions (SUSAR), MedDRA SOC, and MedDRA PT.

9.5. Pregnancies

For any subjects in the Safety population who became pregnant during the study, every attempt will be made to follow these subjects to completion of pregnancy to document the outcome, including information regarding any complications with pregnancy and/or delivery. A listing of pregnancies and outcomes will be presented (Listing 15, Listing 16, Listing 17, Listing 18, and Listing 19).

9.6. Clinical Laboratory Evaluations

Clinical Laboratory measurements will not be graded for severity. Descriptive statistics including mean, std. dev, median, min and max values by time point and change from baseline for each post dose time point will be presented by laboratory parameter. Data will be visualized with box plots and scatter plots.

Summary statistics for Serum creatinine, Magnesium and Liver Function Parameters (AST, ALT, alkaline phosphatase, albumin, total bilirubin) will be reported by parameter and study time point, including change from baseline (Table 13, Table 14, and Table 15). Box plots showing the distribution of change from baseline for all post dose time points with lines connecting the median, quartile 1 and quartile 2 of the change from baseline at each time point will be shown by parameter. (Figure 5, Figure 6, Figure 7, Figure 8, Figure 9, Figure 10, and Figure 11). Scatter plots showing baseline versus 48 h time point will be shown by parameter (Figure 12, Figure 13, Figure 14, Figure 15, Figure 16 , Figure 17, and Figure 18).

Chemistry laboratory measurements scheduled to be collected at baseline and no post dose time points (Potassium, Sodium, Chloride, Bicarbonate, Glucose, Blood Urea Nitrogen) will be summarized in Table 16.

Summary statistics for hematology laboratory parameters (hemoglobin, hematocrit, WBC, WBC differential (neutrophils, lymphocytes, eosinophils, monocytes, basophils), red blood cell count, platelets) will be reported by parameter and study time point, including change from baseline in Table 17. Box plots showing the distribution of change from baseline with lines connecting the median, quartile 1 and quartile 2 of the change from baseline for each of the time points will be shown by parameter (beginning at Figure 19 and concluding with Figure 28).

Urinalysis laboratory parameters (Protein, Glucose, Ketones, Bilirubin, Blood, Nitrites, LCE, Urobilinogen, Specific Gravity, and pH) collected at screening will be summarized in Table 18.

Listing 8Listing 9Listing 10**Vital Signs and Physical Evaluations**

Vital sign measurements included systolic blood pressure, diastolic blood pressure, heart rate, respiratory rate, and temperature. Vital signs were assessed at baseline and 1 h, 24 h, and 48 h post dose. Descriptive statistics including mean, std. dev, median, min and max values by time point and change from baseline for each post dose time point will be presented by assessment in Table 19. Data will be visualized using box plots showing the change from baseline at all post dose study time points with lines connecting the median, quartile 1 and quartile 2 of the change from baseline at each time point (Figure 29, Figure 30, Figure 31, Figure 32, and Figure 33).

Vital signs results will be listed (Listing 11)

A targeted physical examination and examination of specific systems to be guided by symptomatology, will be performed during screening, as well as the SAE follow-up as needed. Results of physical examinations, scheduled and unscheduled, will be presented in Listing 12.

9.8. Concomitant Medications

Concomitant and treatment medications will be collected from 24 hours prior to the start of infusion through 48 hours post start of infusion (total of 72 hours), as well as the SAE follow-up visit as needed. All medications will be coded using the current version of the WHO Drug dictionary. The use of concomitant medications taken 24 hours prior or during the study will be summarized by Anatomical Therapeutic Code ATC 1 and ATC 2 and study time point in Table 21. Individual subject listings will be presented for all prior and concomitant medications (Listing 14).

Receipt of any RRTs from 1-hour post start of infusion through 48 hours post start of infusion will be summarized in Table 20 and listed (Listing 13).

10. PHARMACOKINETICS

PK analysis will be described in the PK SAP.

11. IMMUNOGENICITY

There are no immunogenicity endpoints for this protocol.

12. OTHER ANALYSES

There are no other analyses.

13. REPORTING CONVENTIONS

P-values ≥0.001 and ≤0.999 will be reported to 3 decimal places; p-values less than 0.001 will be reported as "<0.001" The mean, standard deviation, and other statistics will be reported to 1 decimal place greater than the original data. The minimum and maximum will use the same number of decimal places as the original data. Proportions will be presented as 2 decimal places; values greater than zero but <0.01 will be presented as "<0.01". Percentages will be reported to the nearest whole number; values greater than zero but < 1% will be presented as "<1"; values greater than 99% but less than 100% will be reported as >99%. Estimated parameters, not on the same scale as raw observations (e.g. regression coefficients) will be reported to 3 significant figures.

14. TECHNICAL DETAILS

SAS version 9.3 or above will be used to generate all tables, figures and listings.

15. SUMMARY OF CHANGES IN THE CONDUCT OF THE STUDY OR PLANNED ANALYSES

If there are changes to the planned analysis prior to final data lock and after finalization of the Safety SAP, they may be added to the SAP as an addendum. The Safety SAP will not be amended after final data lock.

16. REFERENCES

1. MedCo. Investigator Brochure - Minocin (minocycline) for Injection. In: Company TM, ed. Parsippany, NJ; 2016.
2. Wang, Y., P. R. Jadhav, et al. (2012). "Clarification on Precision Criteria to Derive Sample Size When Designing Pediatric Pharmacokinetic Studies " J Clin Pharmacol 52: 1601-1606

17. LISTING OF TABLES, FIGURES, AND LISTINGS

Table, figure, and listing shells are presented in Appendices 1, 2, and 3.

APPENDICES

APPENDIX 1. TABLE MOCK-UPS

LIST OF TABLES

9.5.1 Efficacy/Immunogenicity and Safety Measurements Assessed and Flow Chart

Table 1: Schedule of Study Procedures

	Screening[1]	Baseline (Hr. 0)	Study Visits (post start of infusion) [17]						Supplemental Visit (as needed)[18]
			1 hr.	4 hrs.	12 hrs.	24 hrs.	36 hrs.	48 hrs.	
Study windows	-	*-30 min*	*+0.5 hr.*	*+1 hr.*	*± 2 hrs.*	*± 2 hrs.*	*± 2 hrs.*	*± 2 hrs.*	-
Clinical Evaluations									
Informed consent	X								
Medical History & Demographics	X								
Height [2]	X[1]								
Weight [2]		X[16]				X		X	
Inclusion / Exclusion Criteria	X								
Targeted Physical Examination [3]	X								X
Vital signs [4]	X	X[16]	X			X		X	X
Collection of RRT's being received			X	X	X	X	X	X	
Concomitant Medications [5]	X	X	X	X	X	X	X	X	X
Collection of SAEs [6]		X	X	X	X	X	X[19]	X[19]	X[19]
Laboratory Evaluations									
Blood chemistry [7]	5 mL[1]	X[14]							
Liver function tests [8]		X[14]						5 mL	
Serum creatinine [9]		5 mL[15]	5 mL	5 mL	5 mL	5 mL	5 mL		
Magnesium									
Hematology[10]	2 mL[1]	2 mL[14]				2 mL		2 mL	
Serum hCG Pregnancy Test [11]	5 mL[1]								
Urinalysis [12]	X[1]								
PK Blood Collection [13]		6 mL[15]	6 mL	6 mL	6 mL	6 mL	6 mL	6 mL	
Study Drug									
Study Drug Administration		X							
APPROXIMATE TOTAL BLOOD	**12 mL**	**13 mL**	**11 mL**	**11 mL**	**11 mL**	**13 mL**	**11 mL**	**13 mL**	

[1]Screening evaluations should be completed within 48 hours prior to enrollment, with exception to the following procedures:
Height can be pulled from the medical records associated with the current hospital admission OR collected within the screening window.
Clinical laboratory evaluations obtained for clinical care purposes (standard of care results) within 48hrs of screening are acceptable for screening. A pregnancy test at any time during the current hospitalization is acceptable for screening.

[2]Height and weight will be measured or calculated as per instructions in the Manual of Procedures, rather than estimated or by report of relative.
[3]A targeted physical examination should include an examination of specific systems, to be guided by symptomatology.
[4]Vital signs should include systolic and diastolic blood pressure (mm Hg), heart rate (HR), respiratory rate, and temperature.
[5]Concomitant medications should be collected from 24 hours prior to start of infusion through 48 hours post start of infusion, as well as SAE follow-up visit as needed
[6]SAEs should be collected from start of infusion through 24 hours post start of infusion.
[7]Blood chemistry panel should include: potassium, sodium, chloride, bicarbonate, glucose, blood urea nitrogen.
[8]Liver function tests should include: AST, ALT, alkaline phosphatase, albumin, total bilirubin.
[9]Serum creatinine may be run as part of the blood chemistry panel if preferred.
[10]Hematology should include: hemoglobin, hematocrit, white blood cell count [WBC], WBC differential, red blood cell count, platelets.
[11]Serum hCG pregnancy test is only required for females of child-bearing potential.
[12]Urinalysis should include: protein, glucose, ketones, bilirubin, blood, nitrites, LCE, urobilinogen, specific gravity, and pH.
[13]Refer to the Manual of Procedures for further details.
[14]A hematology panel, chemistry panel and liver function tests should be repeated if not collected within 8 hours prior to the start of study drug infusion
[15]The Baseline serum creatinine, magnesium and Baseline PK blood draw must be drawn just prior to the start of study drug infusion.
[16]The Baseline weight and vital signs should be collected as close to the start of study drug infusion as possible.
[17]Study windows are as described in the table. For example: 1hr time point with a window of +0.5 hrs, the PK sample can be drawn from 1 to 1.5 hours post start of infusion. At the 24 hour time point, the window is ± 2 hours, so the PK sample can be drawn from 22 to 26 hours post start of infusion.
[18]Supplemental visits should be conducted at the Investigator's discretion and include follow up on any SAEs that are considered related to Study Drug, as well as unscheduled visits. Please see protocol Section 7.6 for details on the SAE Follow-up Visit procedures and Section 7.8 for details on Unscheduled Visits.
[19]SAEs follow-up only after 24 hours

10.2 Protocol Deviations

Table 2: Distribution of Protocol Deviations by Category and Type

Category	Deviation Type	All Subjects (N=X)	
		No. of Subj.	No. of Dev.
Eligibility/enrollment	Any type		
	Did not meet inclusion criterion	x	x
	Met exclusion criterion		
	ICF not signed prior to study procedures		
	Other		
Treatment administration schedule	Any type		
	Out of window visit		
	Missed visit/visit not conducted		
	Missed treatment administration		
	Delayed treatment administration		
	Other		
Follow-up visit schedule	Any type		
	Out of window visit		
	Missed visit/visit not conducted		
	Other		
Protocol procedure/assessment	Any type		
	Incorrect version of ICF signed		
	Blood not collected		
	Urine not collected		
	Other specimen not collected		
	Too few aliquots obtained		
	Specimen result not obtained		
	Required procedure not conducted		
	Required procedure done incorrectly		
	Study product temperature excursion		
	Specimen temperature excursion		
	Other		
Treatment administration	Any type		
	Required procedure done incorrectly		
	Study product temperature excursion		
	Other		
Blinding policy/procedure	Any type		
	Treatment unblinded		
	Other	x	x

Note: N=Enrolled Subjects

14.1.1 Disposition of Subjects

Table 3: Subject Disposition

Subject Disposition	All Subjects (N=X)	
	n	%
Screened	x	--
Enrolled	x	100
Received Any Study Product	x	x
Received Full Dose of Study Product		
Completed All PK Blood Draws		
Completed Follow-up[a]		
PK Evaluable[b]		

Note: N=Enrolled Subjects

[a] Refer to Listing 16.2.1 for reasons that subjects discontinued or terminated early.

[b] To be considered PK evaluable, a subject must have the baseline pre-dose PK sample collected, receive the full infusion of study drug, have at least 3 PK samples collected in the first 12 hours post dose, have at least 1 PK sample collected 24-48 hours post dose, and PK specimens processed per the manual of procedures. Refer to Listing 4 for reasons subjects are excluded from the PK Analysis population.

Table 4: Analysis Populations

[Implementation Note: Although subjects may meet multiple criteria for exclusion, they should be counted under only one reason for exclusion in this table. Priority for assigning reasons for exclusions is consistent with the order in the table.]

Analysis Populations	Reason Subjects Excluded	All Subjects (N=X)	
		n	%
Safety Population	Any Reason	x	x
	Did not receive any dose of study product		
PK Population	Any Reason		
	Missing Pre-Dose PK sample		
	Incomplete infusion of study drug		
	Does not have at least 3 PK samples collected in the first 12 hours post dose		
	Does not have at least 1 PK sample collected 24-48 hours post dose		
	PK specimens not processed per the Manual of Procedures[a]		

Note: N=Enrolled Subjects
[a] Refer to Listing 4 for additional details.

Table 5: Ineligibility Summary of Screen Failures

Inclusion/Exclusion Category	Inclusion/Exclusion Criterion	n[a]	%[b]
Inclusion and Exclusion	Number of subjects failing any eligibility criterion	x	100
Inclusion	Any inclusion criterion	x	x
	[inclusion criterion 1]	x	x
	[inclusion criterion 2]	x	x
	[inclusion criterion 3]	x	x
Exclusion	Any exclusion criterion	x	x
	[exclusion criterion 1]	x	x
	[exclusion criterion 2]	x	x
	[exclusion criterion 3]	x	x

[a] More than one criterion may be marked per subject.
[b] Denominator for percentages is the total number of subjects failing any eligibility criterion.

14.1.2 Demographic Data by Study Group

Table 6: Summary of Categorical Demographic and Baseline Characteristics by Site

[Implementation Note: This table may include other categorical baseline characteristics.]

Variable	Characteristic	[Site 1] (N=X)		[Site 2] (N=X)		All Subjects (N=X)	
		n	%	n	%	n	%
Sex	Male	x	x	x	x	x	x
	Female						
Ethnicity	Not Hispanic or Latino	x	x	x	x	x	x
	Hispanic or Latino						
	Not Reported						
	Unknown						
Race	American Indian or Alaska Native	x	x	x	x	x	x
	Asian						
	Native Hawaiian or Other Pacific Islander						
	Black or African American						
	White						
	Multi-Racial						
	Unknown						

Note: N=Number of subjects in the Safety Population

Table 7: Summary of Continuous Demographic and Baseline Characteristics by Site

Variable	Statistic	[Site 1] (N=X)	[Site 2] (N=X)	All Subjects (N=X)
Age	Mean	x.x	x.x	x.x
	Standard Deviation	x.x	x.x	x.x
	Median	x.x	x.x	x.x
	Minimum	x	x	x
	Maximum	x	x	x
Height	Mean	x.x	x.x	x.x
	Standard Deviation	x.x	x.x	x.x
	Median	x.x	x.x	x.x
	Minimum	x	x	x
	Maximum	x	x	x
Weight	Mean	x.x	x.x	x.x
	Standard Deviation	x.x	x.x	x.x
	Median	x.x	x.x	x.x
	Minimum	x	x	x
	Maximum	x	x	x
BMI	Mean	x.x	x.x	x.x
	Standard Deviation	x.x	x.x	x.x
	Median	x.x	x.x	x.x
	Minimum	x	x	x
	Maximum	x	x	x
CR/CL	Mean	x.x	x.x	x.x
	Standard Deviation	x.x	x.x	x.x
	Median	x.x	x.x	x.x
	Minimum	x	x	x
	Maximum	x	x	x

Note: N= Number of subjects in the Safety Population
CR/CL = Baseline Creatine Clearance calculated using the Cockcroft-Gault formula

14.1.3 Prior and Concurrent Medical Conditions

Table 8: Summary of Subjects with Pre-Existing Medical Conditions by MedDRA System Organ Class

MedDRA System Organ Class	All Subjects (N=X)	
	n	%
Any SOC	x	x
[SOC 1]		
[SOC 2]		

Note: N= Number of subjects in the Safety Population; n = Number of subjects reporting medical history within the specified SOC. A subject is only counted once per SOC.

14.3 Safety Data

14.3.1 Displays of Adverse Events

Table 9: Overall Summary of Serious Adverse Events

	All Subjects (N = x)	
Subjects[a] with	**n**	**%**
At least one SAE[b]	x	x
At least one related SAE		
At least one related Mild (Grade 1) SAE		
At least one related Moderate (Grade 2) SAE		
At least one related Severe (Grade 3) SAE		
At least one SAE leading to early termination		
At least one medically attended SAE		
At least one SUSAR		

Note: N= Number of subjects in the Safety Population; SAE = Serious Adverse Event (unsolicited); SUSAR = Suspected Unexpected Serious Adverse Reaction

[a] Subjects are counted once for each category regardless of the number of events.

[b] A listing of SAEs is included in Table 12.

14.3.1.2 Unsolicited Adverse Events

Table 10: Summary of Unsolicited Serious Adverse Events by MedDRA System Organ Class and Preferred Term

MedDRA System Organ Class	MedDRA Preferred Term	All Subject (N=X)			
		n	%	95% CI	Events
Any SOC	Any PT	x	x	x, x	x
[SOC 1]	Any PT				
	[PT 1]				
	[PT 2]				
[SOC 2]	Any PT				
	[PT 1]				
	[PT 2]				

Note: N = number of subjects in the Safety Population. This table presents number and percentage of subjects. A subject is only counted once per PT/time point.

Table 11: Unsolicited Serious Adverse Events by MedDRA System Organ Class and Preferred Term, Maximum Severity, Relationship, and Treatment Group

MedDRA System Organ Class	Preferred Term	Severity	All Subjects (N = X)					
			Related		Not Related		Total	
			n	%	n	%	n	%
Any SOC	Any PT	Any Severity	x	x	x	x	x	x
		Mild	x	x	x	x	x	x
		Moderate	x	x	x	x	x	x
		Severe	x	x	x	x	x	x
SOC 1	PT 1	Any Severity	x	x	x	x	x	x
		Mild	x	x	x	x	x	x
		Moderate	x	x	x	x	x	x
		Severe	x	x	x	x	x	x
	PT 2	Any Severity	x	x	x	x	x	x
		Mild	x	x	x	x	x	x
		Moderate	x	x	x	x	x	x
		Severe	x	x	x	x	x	x

Note: N = number of subjects in the Safety Population. This table presents number and percentage of subjects. A subject is only counted once per PT/severity.

14.3.2 Listing of Deaths, Other Serious and Significant Adverse Events

Table 12: Listing of Serious Adverse Events

Adverse Event	No. of Days Post Dose (Duration)	No. of Days Post Dose the Event Became Serious	Reason Reported as an SAE	Severity	Relationship to Study Treatment	If Not Related, Alternative Etiology	Action Taken with Study Treatment	Subject Discontinued Due to AE	Outcome	Is the Event a SUSAR?	MedDRA System Organ Class	MedDRA Preferred Term
Subject ID: , AE Number:												
Comments:												
Subject ID: , AE Number:												
Comments:												

14.3.3 Narratives of Deaths, Other Serious and Significant Adverse Events

(not included in SAP, but this is a placeholder for the CSR)

14.3.5 Displays of Laboratory Results

14.3.5.1 Chemistry Results

Table 13: Chemistry Laboratory Results and Change from Baseline, Summary Statistics by Parameter and Time Point – Serum Creatinine

		Measurement				Change from Baseline			
Time Point	**n**	**Mean**	**SD**	**Median**	**Min, Max**	**Mean**	**SD**	**Median**	**Min, Max**
Baseline	x	x.x	x.x	x.x	x.x, x.x	-	-	-	-
1 h Post start of infusion	x	x.x	x.x	x.x	x.x, x.x	x.x	x.x	x.x	x.x, x.x
4 h Post start of infusion									
12 h Post start of infusion									
24 h Post start of infusion									
36 h Post start of infusion									
48 h Post start of infusion									

Note: n = Number of subjects in the Safety Population with clinical safety labs results at the respective study time point.

Table 14: Chemistry Laboratory Results and Change from Baseline, Summary Statistics by Parameter and Time Point – Magnesium

		Measurement				Change from Baseline			
Time Point	**n**	**Mean**	**SD**	**Median**	**Min, Max**	**Mean**	**SD**	**Median**	**Min, Max**
Baseline	x	x.x	x.x	x.x	x.x, x.x	-	-	-	-
24 h Post start of infusion	x	x.x	x.x	x.x	x.x, x.x	x.x	x.x	x.x	x.x, x.x
48 h Post start of infusion									

Note: n = Number of subjects in the Safety Population with clinical safety labs results at the respective study time point.

Table 15: Chemistry Laboratory Results and Change from Baseline, Summary Statistics by Parameter and Time Point – Liver Function Tests

		Measurement				Change from Baseline			
Time Point	**n**	**Mean**	**SD**	**Median**	**Min, Max**	**Mean**	**SD**	**Median**	**Min, Max**
AST									
Baseline	x	x.x	x.x	x.x	x.x, x.x	-	-	-	-
48 h Post start of infusion	x	x.x	x.x	x.x	x.x, x.x	x.x	x.x	x.x	x.x, x.x
ALT									
Baseline	x	x.x	x.x	x.x	x.x, x.x	-	-	-	-
48 h Post start of infusion	x	x.x	x.x	x.x	x.x, x.x	x.x	x.x	x.x	x.x, x.x
Alkaline Phosphatase									
Baseline	x	x.x	x.x	x.x	x.x, x.x	-	-	-	-
48 h Post start of infusion	x	x.x	x.x	x.x	x.x, x.x	x.x	x.x	x.x	x.x, x.x
Albumin									
Baseline	x	x.x	x.x	x.x	x.x, x.x	-	-	-	-
48 h Post start of infusion	x	x.x	x.x	x.x	x.x, x.x	x.x	x.x	x.x	x.x, x.x
Total Bilirubin									
Baseline	x	x.x	x.x	x.x	x.x, x.x	-	-	-	-
48 h Post start of infusion	x	x.x	x.x	x.x	x.x, x.x	x.x	x.x	x.x	x.x, x.x

Note: n = Number of subjects in the Safety Population with clinical safety labs results at the respective study time point.

Table 16: Chemistry Laboratory Results by Parameter Collected at Baseline – Blood Chemistry Panel

Baseline Measurement	n	Mean	SD	Median	Min, Max
Potassium	x	x.x	x.x	x.x	x.x, x.x
Sodium					
Chloride					
Bicarbonate					
Glucose					
Blood Urea Nitrogen					

Note: n = Number of subjects in the Safety Population with clinical safety labs results at baseline. Laboratory measurements not scheduled to be collected at any post dose time points.

14.3.5.2 Hematology Results

Table 17: Hematology Laboratory Summary Statistics and Change from Baseline by Parameter and Time Point

		Measurement				Change from Baseline			
Time Point	**n**	**Mean**	**SD**	**Median**	**Min, Max**	**Mean**	**SD**	**Median**	**Min, Max**
Hemoglobin									
Baseline	x	x.x	x.x	x.x	x.x, x.x	-	-	-	-
24 h Post start of infusion	x	x.x	x.x	x.x	x.x, x.x	x.x	x.x	x.x	x.x, x.x
48 h Post start of infusion									
Hematocrit									
Baseline	x	x.x	x.x	x.x	x.x, x.x	-	-	-	-
24 h Post start of infusion	x	x.x	x.x	x.x	x.x, x.x	x.x	x.x	x.x	x.x, x.x
48 h Post start of infusion									
WBS									
Baseline	x	x.x	x.x	x.x	x.x, x.x	-	-	-	-
24 h Post start of infusion	x	x.x	x.x	x.x	x.x, x.x	x.x	x.x	x.x	x.x, x.x
48 h Post start of infusion									
Neutrophils									
Baseline	x	x.x	x.x	x.x	x.x, x.x	-	-	-	-
24 h Post start of infusion	x	x.x	x.x	x.x	x.x, x.x	x.x	x.x	x.x	x.x, x.x
48 h Post start of infusion									
Lymphocytes									
Baseline	x	x.x	x.x	x.x	x.x, x.x	-	-	-	-
24 h Post start of infusion	x	x.x	x.x	x.x	x.x, x.x	x.x	x.x	x.x	x.x, x.x
48 h Post start of infusion									
Eosinophils									
Baseline	x	x.x	x.x	x.x	x.x, x.x	-	-	-	-
24 h Post start of infusion	x	x.x	x.x	x.x	x.x, x.x	x.x	x.x	x.x	x.x, x.x
48 h Post start of infusion									
Monocytes									
Baseline	x	x.x	x.x	x.x	x.x, x.x	-	-	-	-
24 h Post start of infusion	x	x.x	x.x	x.x	x.x, x.x	x.x	x.x	x.x	x.x, x.x
48 h Post start of infusion									
Basophils									
Baseline	x	x.x	x.x	x.x	x.x, x.x	-	-	-	-
24 h Post start of infusion	x	x.x	x.x	x.x	x.x, x.x	x.x	x.x	x.x	x.x, x.x
48 h Post start of infusion									

		Measurement				Change from Baseline			
Time Point	**n**	**Mean**	**SD**	**Median**	**Min, Max**	**Mean**	**SD**	**Median**	**Min, Max**
Platelets									
Baseline	x	x.x	x.x	x.x	x.x, x.x	-	-	-	-
24 h Post start of infusion	x	x.x	x.x	x.x	x.x, x.x	x.x	x.x	x.x	x.x, x.x
48 h Post start of infusion									
Red Blood Cell Count									
Baseline	x	x.x	x.x	x.x	x.x, x.x	-	-	-	-
24 h Post start of infusion	x	x.x	x.x	x.x	x.x, x.x	x.x	x.x	x.x	x.x, x.x
48 h Post start of infusion									

Note: n = Number of subjects in the Safety Population with clinical safety labs results at the respective study time point.

14.3.5.3 Urinalysis Results

Table 18: Urinalysis Laboratory Results by Parameter at Collected Screening

[Repeat for each Urinalysis Laboratory Parameter and a table for each Parameter's Change from Baseline; number each table separately.]

Screening Measurement	N	Mean	SD	Median	Min, Max
Protein	x	x.x	x.x	x.x	x.x, x.x
Glucose					
Ketones					
Bilirubin					
Blood					
Nitrites					
LCE					
Urobilinogen					
Specific Gravity					
pH					

Note: n = Number of subjects in the Safety Population with clinical safety labs results at screening. Urinalysis laboratory parameters only measured at screening.

14.3.6 Displays of Vital Signs

Table 19: Vital Signs Summary Statistics and Change from Baseline by Assessment and Time Point

		Measurement				Change from Baseline			
Time Point	**n**	**Mean**	**SD**	**Median**	**Min, Max**	**Mean**	**SD**	**Median**	**Min, Max**
Systolic Blood Pressure									
Baseline	x	x.x	x.x	x.x	x.x, x.x	-	-	-	-
1 h Post start of infusion	x	x.x	x.x	x.x	x.x, x.x	x.x	x.x	x.x	x.x, x.x
24 h Post start of infusion									
48 h Post start of infusion									
Diastolic Blood Pressure									
Baseline	x	x.x	x.x	x.x	x.x, x.x	-	-	-	-
1 h Post start of infusion	x	x.x	x.x	x.x	x.x, x.x	x.x	x.x	x.x	x.x, x.x
24 h Post start of infusion									
48 h Post start of infusion									
Heart Rate									
Baseline	x	x.x	x.x	x.x	x.x, x.x	-	-	-	-
1 h Post start of infusion	x	x.x	x.x	x.x	x.x, x.x	x.x	x.x	x.x	x.x, x.x
24 h Post start of infusion									
48 h Post start of infusion									
Respiration Rate									
Baseline	x	x.x	x.x	x.x	x.x, x.x	-	-	-	-
1 h Post start of infusion	x	x.x	x.x	x.x	x.x, x.x	x.x	x.x	x.x	x.x, x.x
24 h Post start of infusion									
48 h Post start of infusion									
Temperature									
Baseline	x	x.x	x.x	x.x	x.x, x.x	-	-	-	-
1 h Post start of infusion	x	x.x	x.x	x.x	x.x, x.x	x.x	x.x	x.x	x.x, x.x
24 h Post start of infusion									
48 h Post start of infusion									

Note: n = Number of subjects in the Safety Population with assessment collected at the respective study time point.

14.4 Summary of Concomitant Medications

Table 20: Number and Percentage of Subjects with Renal Replacement Therapies (RRT)

Type of Therapy	All Subjects (N=X)	
	n	%
Any RRT	x	x
Slow Continuous Ultrafiltration	x	x
Continuous Veno-Venous Hemofiltration		
Continuous Veno-Venous Hemodialysis		
….		

N= Number of subjects in the Safety Population, n=Number of subjects reporting RRT

Table 21: Number and Percentage of Subjects with Prior and Concurrent Medications by WHO Drug Classification by Time Point

WHO Drug Code Level 1, Anatomic Group	WHO Drug Code Level 2, Therapeutic Subgroup	All Subjects (N = X)															
		<24 Hours Prior to Infusion		Start to 1.5 Hours Post Infusion		>1.5 to 5 Hours Post Infusion		> 5 to 14 Hours Post Infusion		>14 to 26 Hours Post Infusion		>26 to 38 Hours Post Infusion		> 38 to 50 Hours Post Infusion		Any Time Period	
		n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Any Level 1 Codes	Any Level 2 Codes	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
[ATC Level 1 - 1]	Any [ATC 1 – 1]																
	[ATC 2 - 1]																
	[ATC 2 - 2]																
	[ATC 2 - 3]																
[ATC Level 1 – 2]	[ATC 2 - 1]																
	[ATC 2 - 2]																
	[ATC 2 - 3]																

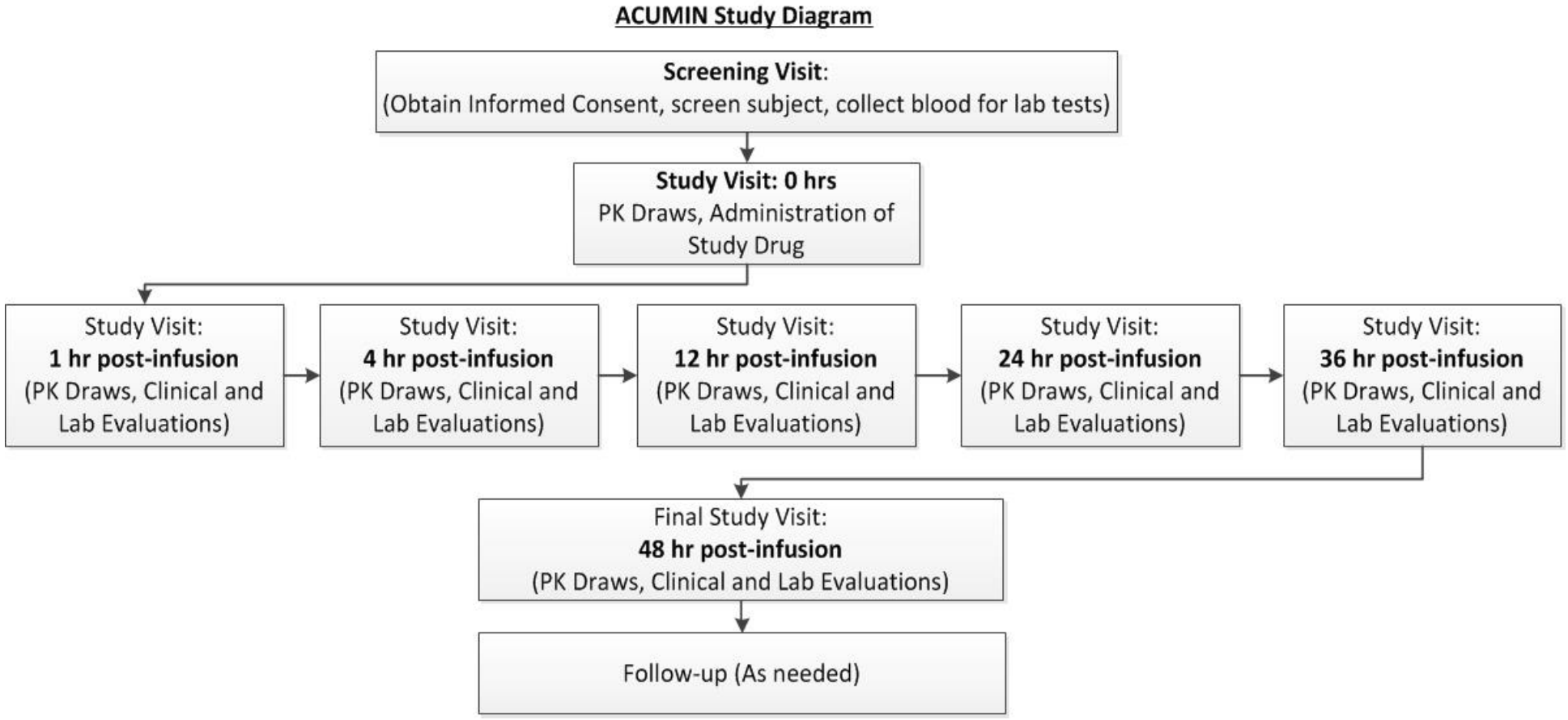
Note: N= Number of subjects in the Safety Population, n=Number of subjects reporting taking at least one medication in the specific WHO Drug Class for that time period.

APPENDIX 2. FIGURE MOCK-UPS

LIST OF FIGURES

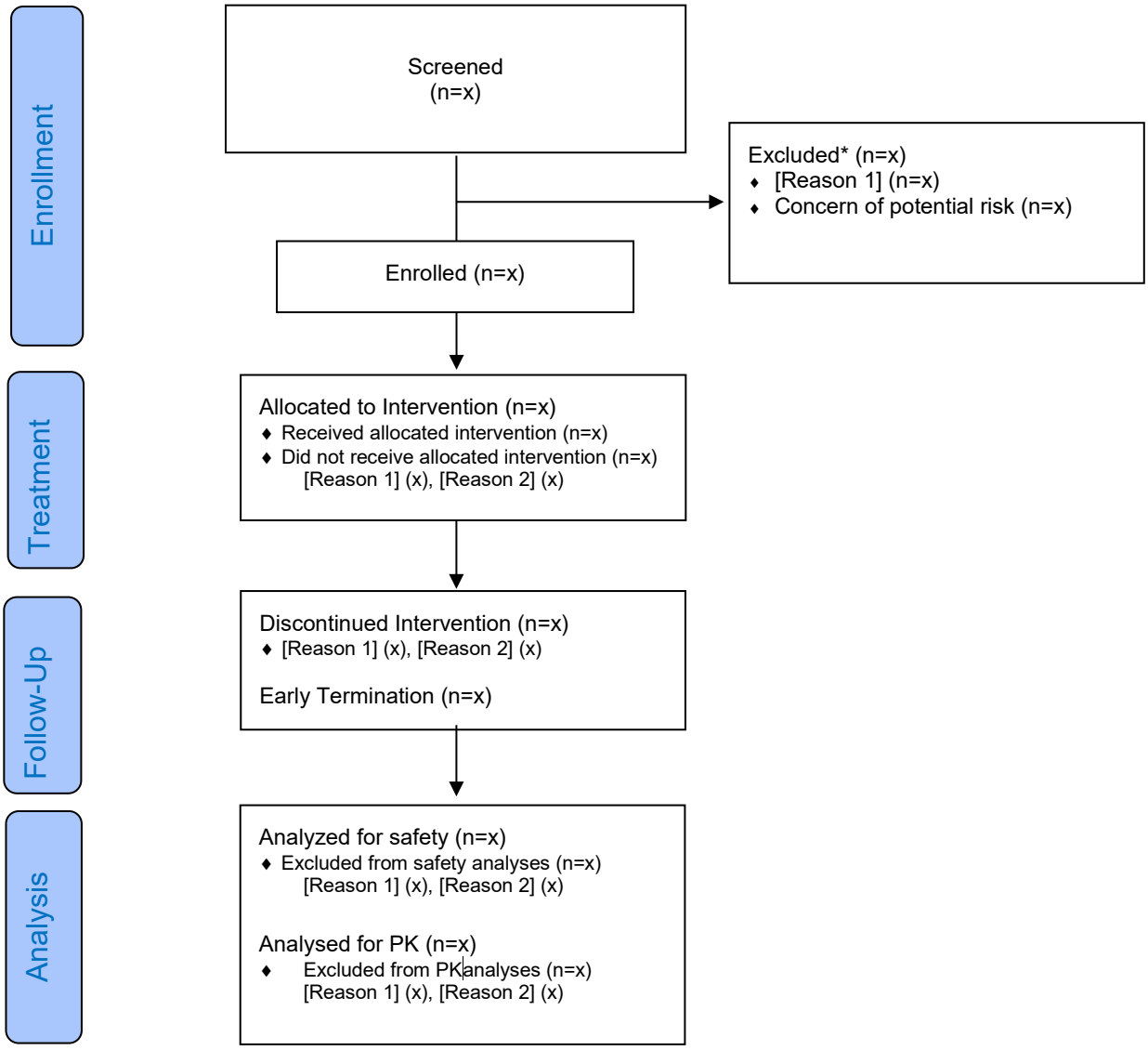
9.1 Overall Study Design and Plan Description

Figure 1: Study Design



10.1 Disposition of Subjects

Figure 2: Study Flow Diagram



14.3.1.2 Unsolicited Serious Adverse Events

Figure 3: Frequency of Related Serious Adverse Events by MedDRA System Organ Class and Severity

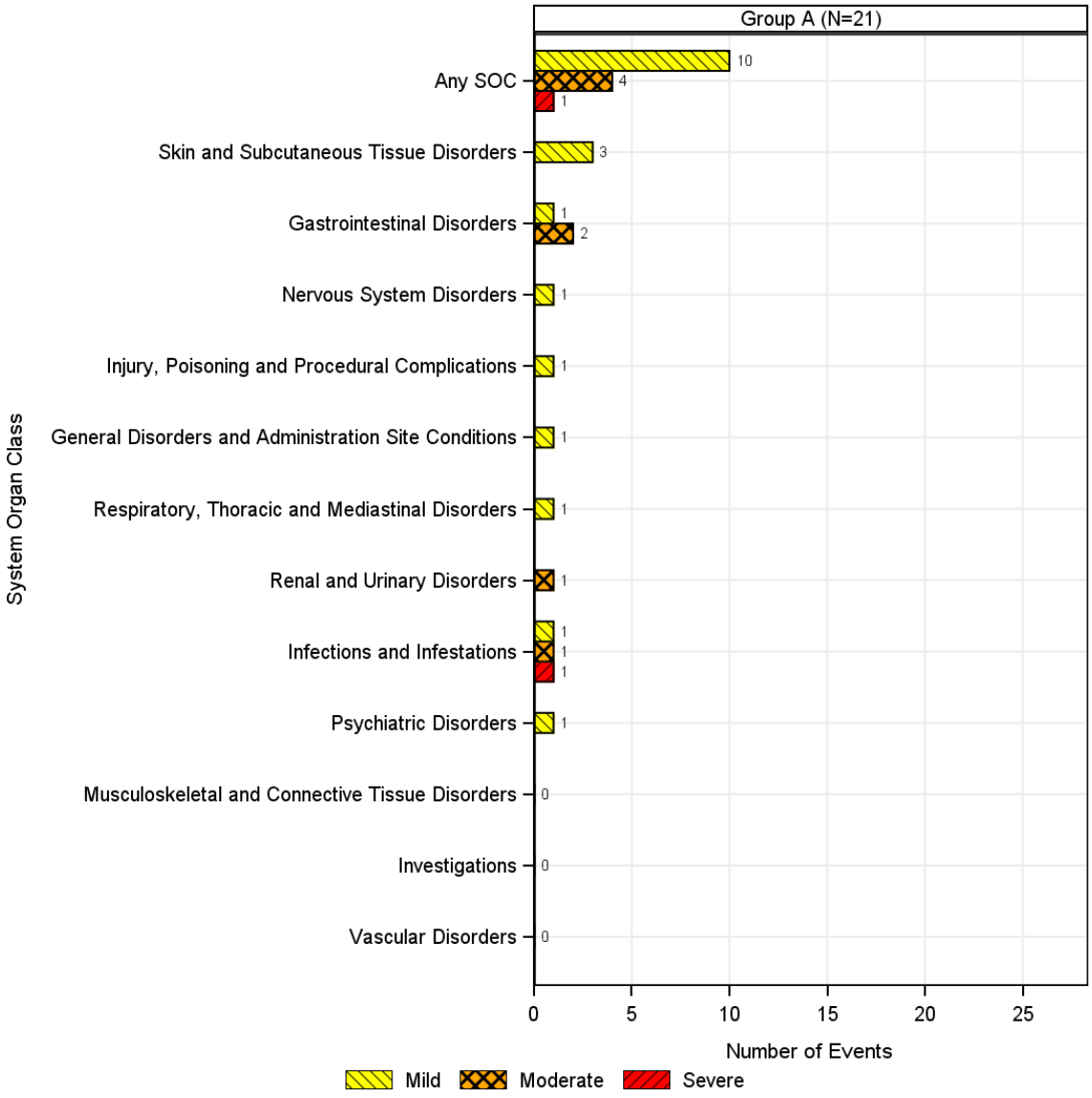
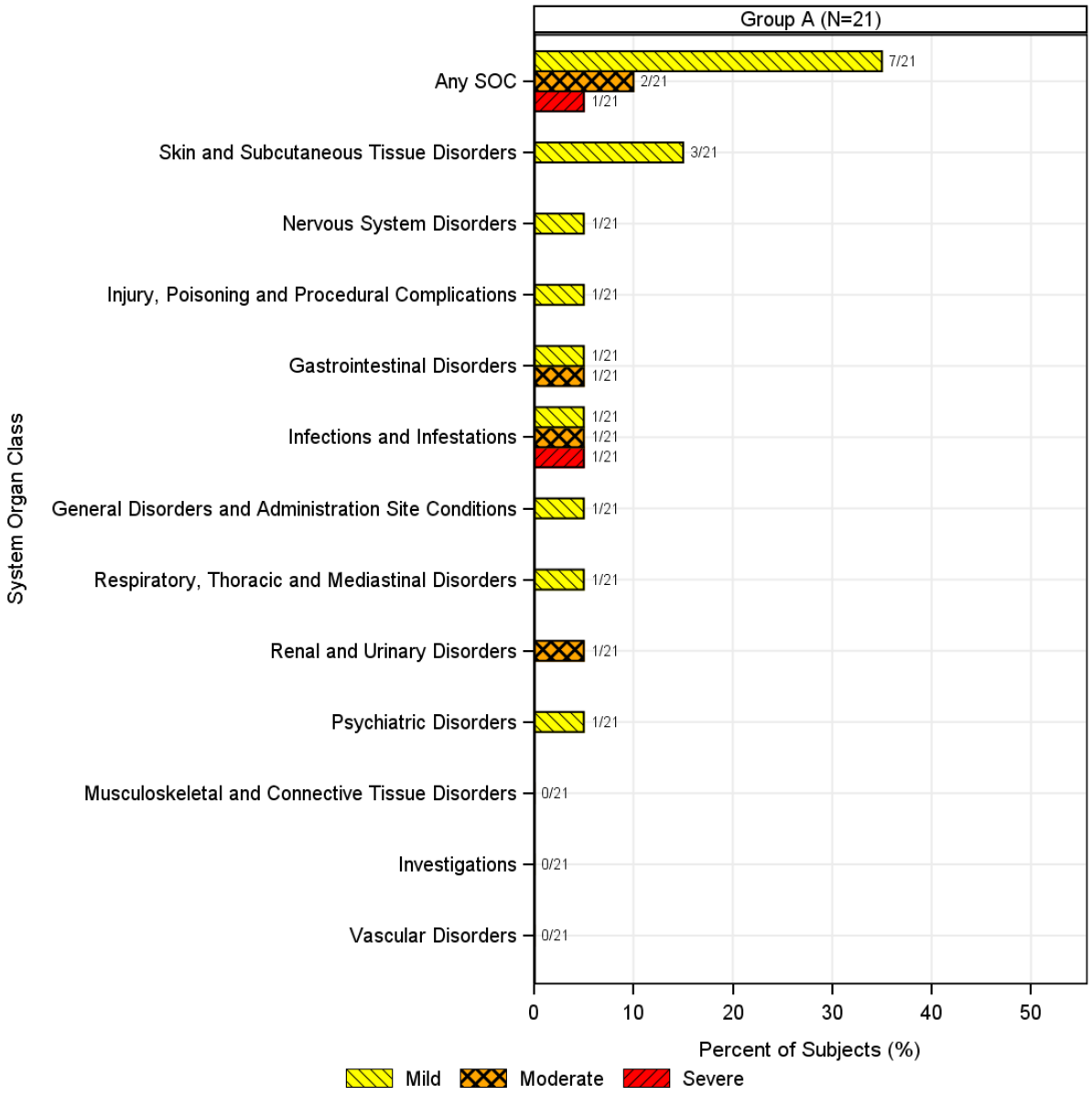


Figure 4: Incidence of Related Serious Adverse Events by MedDRA System Organ Class and Maximum Severity



14.3.5 Displays of Laboratory Results

14.3.5.1 Chemistry Results

Figure 5: Laboratory Results by Scheduled Time Points: Box Plot of Change from Baseline by Laboratory Parameter – Serum Creatine

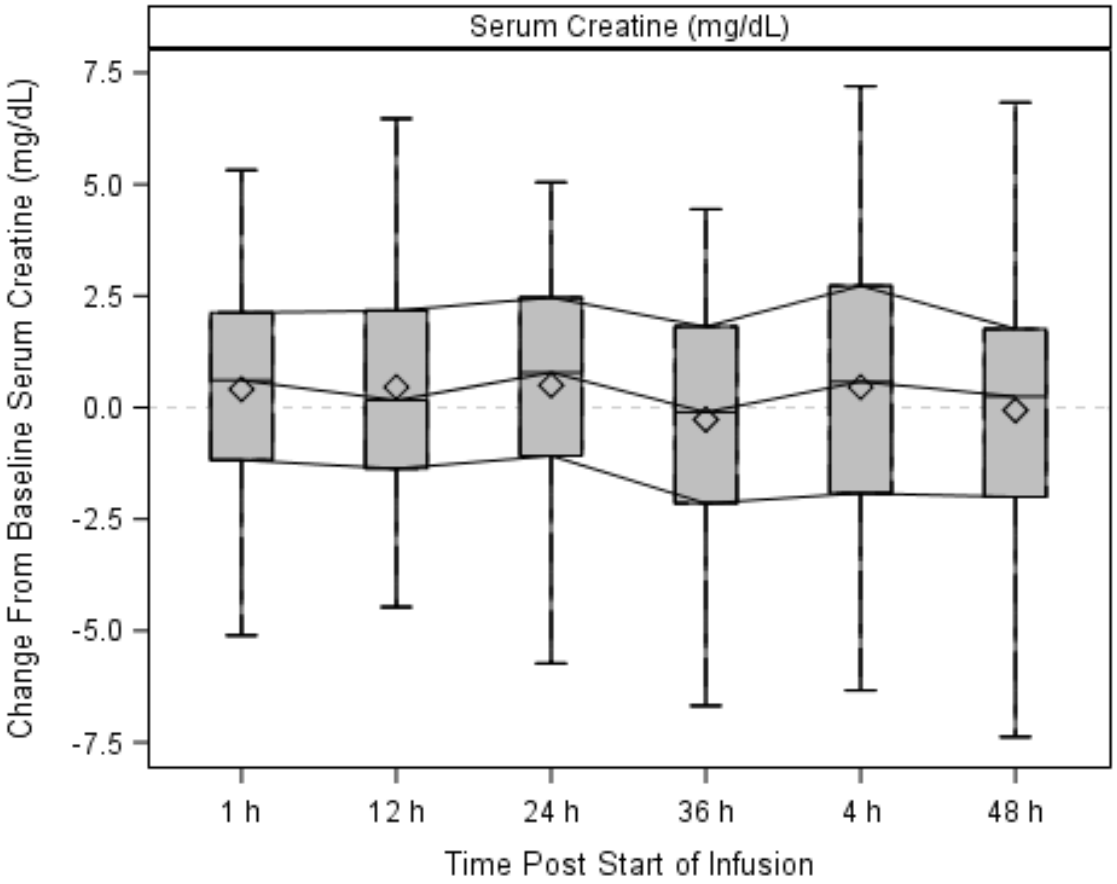


Figure 6: Laboratory Results by Scheduled Time Points: Box Plot of Change from Baseline by Laboratory Parameter – Magnesium

This figure will repeat Figure 5 for Magnesium.

Figure 7: Laboratory Results by Scheduled Time Points: Box Plot of Change from Baseline by Laboratory Parameter – AST

This figure will repeat Figure 5 for AST.

Figure 8: Laboratory Results by Scheduled Time Points: Box Plot of Change from Baseline by Laboratory Parameter – ALT

This figure will repeat Figure 5 for ALT.

Figure 9: Laboratory Results by Scheduled Time Points: Box Plot of Change from Baseline by Laboratory Parameter – Alkaline Phosphatase

This figure will repeat Figure 5 for Alkaline Phosphatase.

Figure 10: Laboratory Results by Scheduled Time Points: Box Plot of Change from Baseline by Laboratory Parameter – Albumin

This figure will repeat Figure 5 for Albumin.

Figure 11: Laboratory Results by Scheduled Time Points: Box Plot of Change from Baseline by Laboratory Parameter – Total Bilirubin

This figure will repeat Figure 5 for Total Bilirubin.

Figure 12: Laboratory Results: Scatter Plot of Baseline Compared with 48 h Post Dose – Serum Creatine

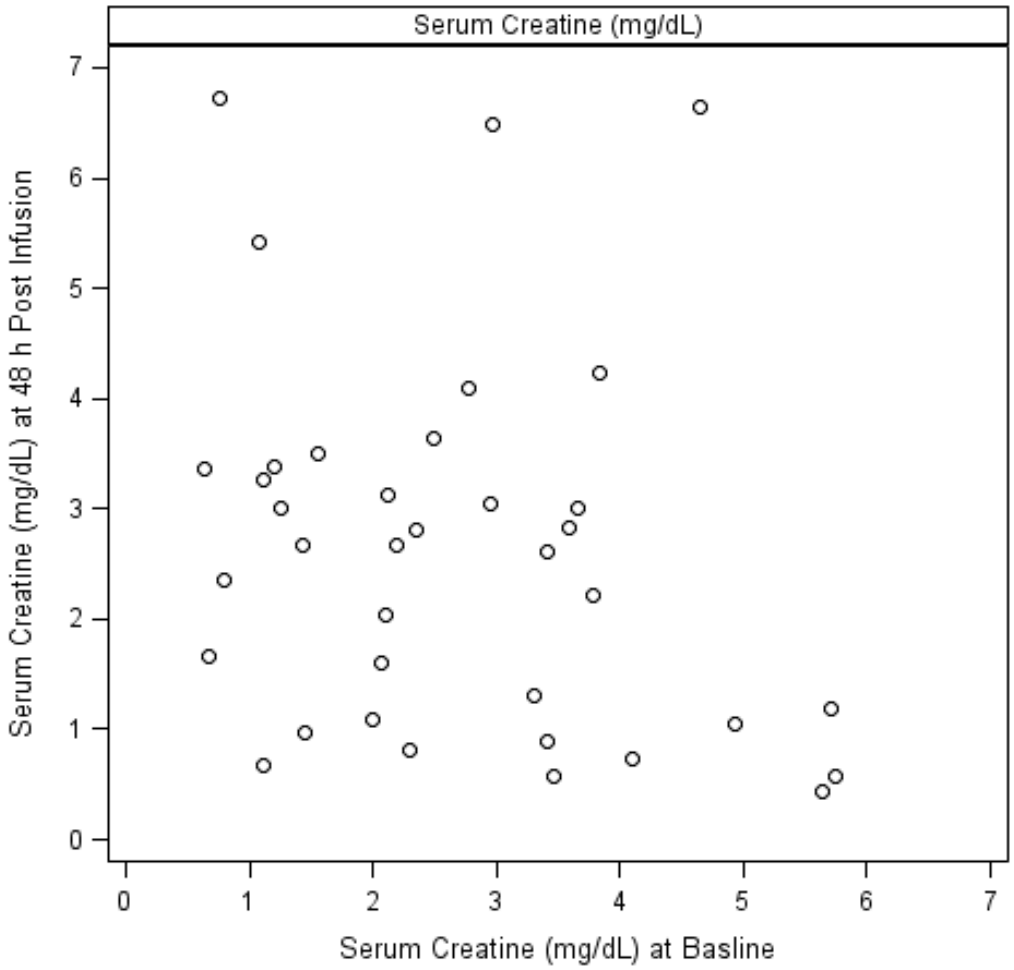


Figure 13: Laboratory Results: Scatter Plot of Baseline Compared with 48 h Post Dose – Magnesium

This figure will repeat Figure 12 for Magnesium.

Figure 14: Laboratory Results: Scatter Plot of Baseline Compared with 48 h Post Dose – AST

This figure will repeat Figure 12 for AST.

Figure 15: Laboratory Results: Scatter Plot of Baseline Compared with 48 h Post Dose – ALT

This figure will repeat Figure 12 for ALT.

Figure 16: Laboratory Results: Scatter Plot of Baseline Compared with 48 h Post Dose – Alkaline Phosphatase

This figure will repeat Figure 12 for Alkaline Phosphatase.

Figure 17: Laboratory Results: Scatter Plot of Baseline Compared with 48 h Post Dose – Albumin

This figure will repeat Figure 12 for Albumin.

Figure 18: Laboratory Results: Scatter Plot of Baseline Compared with 48 h Post Dose – Total Bilirubin

This figure will repeat Figure 12 for Total Bilirubin.

14.3.5.2 Hematology Results

Figure 19: Laboratory Results by Scheduled Time Points: Box Plot of Change from Baseline by Laboratory Parameter – Hemoglobin

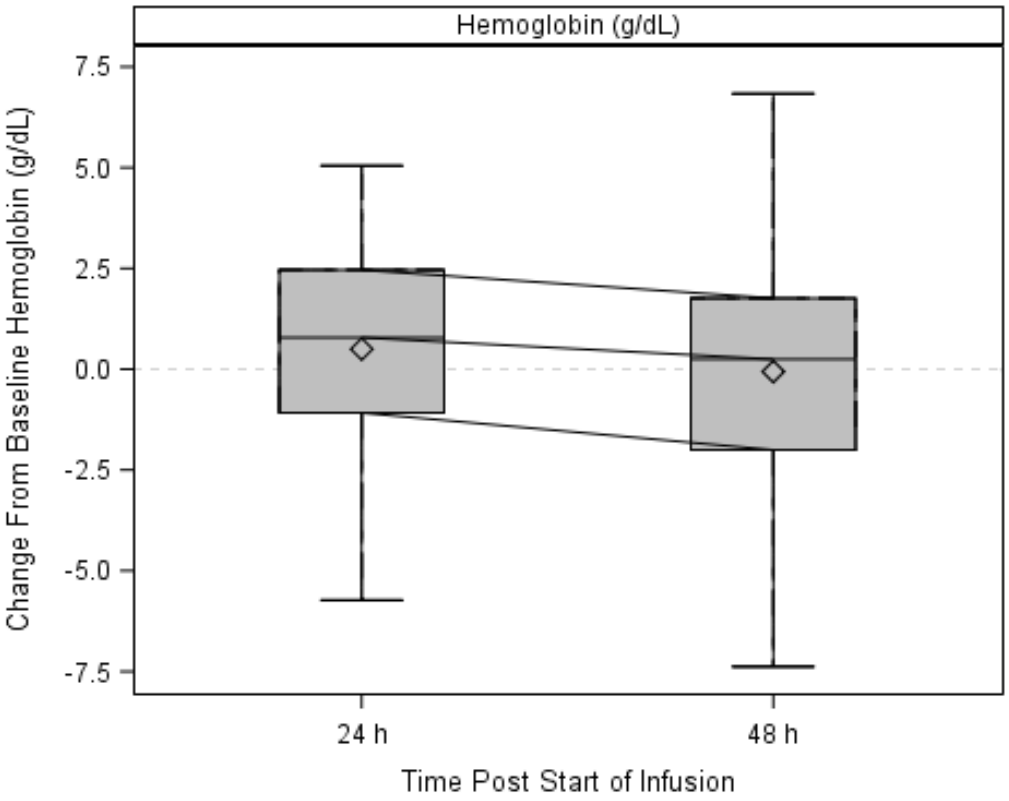


Figure 20: Laboratory Results by Scheduled Time Points: Box Plot of Change from Baseline by Laboratory Parameter – Hematocrit

This figure will repeat Figure 19 for Hematocrit.

Figure 21: Laboratory Results by Scheduled Time Points: Box Plot of Change from Baseline by Laboratory Parameter – WBC

This figure will repeat Figure 19 for WBC.

Figure 22: Laboratory Results by Scheduled Time Points: Box Plot of Change from Baseline by Laboratory Parameter – Neutrophils

This figure will repeat Figure 19 for Neutrophils.

Figure 23: Laboratory Results by Scheduled Time Points: Box Plot of Change from Baseline by Laboratory Parameter – Lymphocytes

This figure will repeat Figure 19 for Lymphocytes.

Figure 24: Laboratory Results by Scheduled Time Points: Box Plot of Change from Baseline by Laboratory Parameter – Eosinophils

This figure will repeat Figure 19 for Eosinophils.

Figure 25: Laboratory Results by Scheduled Time Points: Box Plot of Change from Baseline by Laboratory Parameter – Monocytes

This figure will repeat Figure 19 for Monocytes.

Figure 26: Laboratory Results by Scheduled Time Points: Box Plot of Change from Baseline by Laboratory Parameter – Basophils

This figure will repeat Figure 19 for Basophils.

Figure 27: Laboratory Results by Scheduled Time Points: Box Plot of Change from Baseline by Laboratory Parameter – Red Blood Cell Count

This figure will repeat Figure 19 for Red Blood Cell Count.

Figure 28: Laboratory Results by Scheduled Time Points: Box Plot of Change from Baseline by Laboratory Parameter – Platelets

This figure will repeat Figure 19 for Platelets.

14.3.6 Displays of Vital Signs

Figure 29: Vital Signs by Scheduled Time Point: Box Plot of Change from Baseline by Assessment – Systolic Blood Pressure

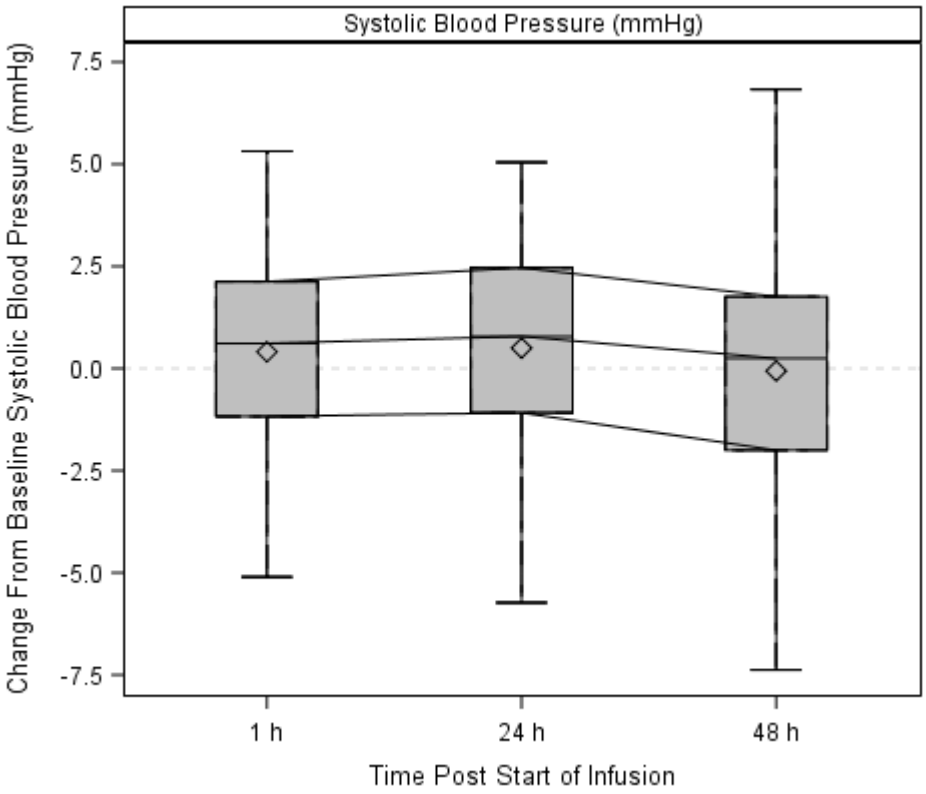


Figure 30: Vital Signs by Scheduled Visits: Box Plot of Change from Baseline by Assessment – Diastolic Blood Pressure

This figure will repeat Figure 29 for Diastolic Blood Pressure.

Figure 31: Vital Signs by Scheduled Visits: Box Plot of Change from Baseline by Assessment – Heart Rate

This figure will repeat Figure 29 for Heart Rate.

Figure 32: Vital Signs by Scheduled Visits: Box Plot of Change from Baseline by Assessment – Respiratory Rate

This figure will repeat Figure 29 for Respiratory Rate.

Figure 33: Vital Signs by Scheduled Visits: Box Plot of Change from Baseline by Assessment – Oral Temperature

This figure will repeat Figure 29 for Oral Temperature.

APPENDIX 3. LISTINGS MOCK-UPS

LISTINGS

16.1.6 Listing of Subjects Receiving Investigational Product

(not included in SAP, but this is a placeholder for the CSR)

16.2 Database Listings by Subject

16.2.1 Discontinued Subjects

Listing 1: 16.2.1 Early Terminations or Discontinued Subjects

Subject ID	Category	Reason for Early Termination or Treatment Discontinuation	Study Day

16.2.2 Protocol Deviations

Listing 2: 16.2.2.1: Subject-Specific Protocol Deviations

Subject ID	DV Number	Deviation	Deviation Category	Study Day	Reason for Deviation	Deviation Resulted in AE?	Deviation Resulted in Subject Termination?	Deviation Affected Product Stability?	Deviation Resolution	Comments

Listing 3: 16.2.2.2: Non-Subject-Specific Protocol Deviations

Site	Start Date	Deviation	End Date	Reason for Deviation	Deviation Resulted in Subject Termination?	Deviation Affected Product Stability?	Deviation Category	Deviation Resolution	Comments

16.2.3 Subjects Excluded from the Efficacy Analysis

Listing 4: 16.2.3: Subjects Excluded from Analysis Populations

Subject ID	Analyses in which Subject is Included	Analyses from which Subject is Excluded	Results Available?	Reason Subject Excluded
	[e.g., Safety, PK]	[e.g., Safety, PK, Day x]		

Note: "Yes" in the "Results available" column indicates that available data were removed from the analysis. "No" indicates that no data were available for inclusion in the analysis.

16.2.4 Demographic Data

Listing 5: 16.2.4.1: Demographic Data

Subject ID	Sex	Age at Enrollment (years)	Ethnicity	Race

Listing 6: 16.2.4.2: Pre-Existing and Concurrent Medical Conditions

Subject ID	MH Number	Medical History Term	Condition Start Day	Condition End Day	MedDRA System Organ Class	MedDRA Preferred Term

16.2.5 Compliance and/or Drug Concentration Data

Listing 7: 16.2.5: Drug Concentration Data for Subjects with Incomplete Infusion

[Implementation note: only include subjects who did not receive the full infusion of Minocin IV (< 200mg)]

Subject ID	Actual Dosage Administered

16.2.8 Individual Laboratory Measurements

Listing 8: 16.2.8.1: Clinical Laboratory Results – Chemistry

Subject ID	Planned Time Point	Actual Study Day	Sex	Age (years)	Laboratory Parameter (Units)

Listing 9: 16.2.8.2: Clinical Laboratory Results – Hematology

Subject ID	Planned Time Point	Actual Study Day	Sex	Age (years)	Laboratory Parameter (Units)

Listing 10: 16.2.8.3: Clinical Laboratory Results – Urinalysis

Subject ID	Planned Time Point	Actual Study Day	Sex	Age (years)	Laboratory Parameter (Units)

16.2.9 Vital Signs and Physical Exam Findings

Listing 11: 16.2.9.1: Vital Signs

Subject ID	Planned Time Point	Actual Study Day	Temperature (°C)	Systolic Blood Pressure (mmHg)	Diastolic Blood Pressure (mmHg)	Heart Rate (beats/min)	Respiratory Rate (breaths/min)	Weight (kg)	Height (cm)

Listing 12: 16.2.9.2: Physical Exam Findings

Subject ID	Planned Time Point	Actual Study Day	Body System	Abnormal Finding	Reported as an AE? (AE Description; Number)

Listing 13: 16.2.9.3: Receipt of Renal Replacement Therapies (RRT)

Subject ID	Study Time Point	Type of Therapy

16.2.10 Concomitant Medications

Listing 14: 16.2.10: Concomitant Medications

Subject ID	CM Number	Medication	Indication	Time Period Relative to Infusion	ATC Level 1 (ATC Level 2)

16.2.11 Pregnancy Reports

Listing 15: 16.2.11.1: Pregnancy Reports – Maternal Information

Subject ID	Pregnancy Number	Study Day Corresponding to Estimated Date of Conception	Source of Maternal Information	Pregnancy Status	Mother's Pre-Pregnancy BMI	Mother's Weight Gain During Pregnancy	Tobacco, Alcohol, or Drug Use During Pregnancy?	Medications During Pregnancy?	Maternal Complications During Pregnancy?	Maternal Complications During Labor, Delivery, or Post-Partum?

Note: Maternal Complications are included in the Adverse Event listing. Medications taken during pregnancy are included in the Concomitant Medications Listing.

Listing 16: 16.2.11.2: Pregnancy Reports – Gravida and Para

			Live Births												
Subject ID	Pregnancy Number	Gravida	Extremely PB[a]	Very Early PB[a]	Early PB[a]	Late PB[a]	Early TB[a]	Full TB[b]	Late TB[b]	Post TB[b]	Still Births	Spontaneous Abortion/ Miscarriage	Elective Abortions	Therapeutic Abortions	Major Congenital Anomaly with Previous Pregnancy?

Note: Gravida includes the current pregnancy, para events do not.

[a]Preterm Birth

[b]Term Birth

Listing 17: 16.2.11.3: Pregnancy Reports – Live Birth Outcomes

Subject ID	Pregnancy Number	Fetus Number	Pregnancy Outcome (for this Fetus)	Fetal Distress During Labor and Delivery?	Delivery Method	Gestational Age at Live Birth	Size for Gestational Age	Apgar Score, 1 minute	Apgar Score, 5 minutes	Cord pH	Congenital Anomalies?	Illnesses/ Hospitalizations within 1 Month of Birth?

Note: Congenital Anomalies are included in the Adverse Event listing.

Listing 18: 16.2.11.4: Pregnancy Reports – Still Birth Outcomes

Subject ID	Date of Initial Report	Fetus Number	Pregnancy Outcome (for this Fetus)	Fetal Distress During Labor and Delivery?	Delivery Method	Gestational Age at Still Birth	Size for Gestational Age	Cord pH	Congenital Anomalies?	Autopsy Performed?	If Autopsy, Etiology for Still Birth Identified?

Listing 19: 16.2.11.5: Pregnancy Reports – Spontaneous, Elective, or Therapeutic Abortion Outcomes

Subject ID	Date of Initial Report	Fetus Number	Pregnancy Outcome (for this Fetus)	Gestational Age at Termination	Abnormality in Product of Conception?	Reason for Therapeutic Abortion

DMID Protocol: 16-0011
Date: June 20, 2019
Version: 2.0

DATA ANALYSIS PLAN FOR THE POPULATION PHARMACOKINETIC AND PHARMACOKINETIC-PHARMACODYNAMIC TARGET ATTAINMENT ANALYSIS OF MINOCYCLINE IN CRITICALLY-ILL ADULTS, WITH ILLNESS KNOWN OR SUSPECTED TO BE CAUSED BY INFECTION WITH GRAM-NEGATIVE BACTERIA

Data Coordinating Center

The Emmes Corporation

401 North Washington Street, Suite 700

Rockville, MD

DATA ANALYSIS PLAN FOR THE POPULATION PHARMACOKINETIC AND PHARMACOKINETIC-PHARMACODYNAMIC TARGET ATTAINMENT ANALYSIS OF MINOCYCLINE IN CRITICALLY-ILL ADULTS, WITH ILLNESS KNOWN OR SUSPECTED TO BE CAUSED BY INFECTION WITH GRAM-NEGATIVE BACTERIA

SIGNATURE PAGE

SPONSOR:	Division of Microbiology and Infectious Diseases National Institute of Allergy and Infectious Diseases National Institutes of Health
STUDY TITLE:	A Phase IV Open Label Pharmacokinetic Study of Minocycline for Injection Following a Single Infusion in Critically-Ill Adults (ACUMIN)
PROTOCOL NUMBER:	DMID 16-0011

Pharmacometrics Scientist: Scott Van Wart, M.S., Ph.D. Vice President and CSO Enhanced Pharmacodynamics, LLC	Date
Principal Investigator: Richard G. Wunderink, MD Northwestern Memorial Hospital	Date
Co-Investigator: Thomas Lodise, Pharm.D., Ph.D. Albany College of Pharmacy and Health Sciences	Date
DMID Scientific Lead: Jane Knisely, Ph.D.	Date
Statistician: Thomas Conrad The Emmes Corporation	Date

REPORT CONTENTS

FIGURE LISTING

APPENDIX LISTING

ABBREVIATION LISTING

Abbreviation	Definition
ADD	Additive
AIC	Akaike's Information Criterion
Alpha, α	Level of significance or distribution-phase elimination rate constant (hr^{-1})
AUC_{0-24}	Area under the plasma minocycline concentration-time curve from 0 to 24 hours after a dose (mg•hr/L)
AUC_{0-last}	Area under the plasma minocycline concentration-time curve from 0 to the time of the last quantifiable sample after a dose (mg•hr/L)
$AUC_{0-\infty}$	Area under the plasma minocycline concentration-time curve from 0 to infinity after a dose (mg•hr/L)
Beta, β	Elimination-phase rate constant (hr^{-1})
BMI	Body mass index (kg/m^2)
BSA	Body surface area (m^2)
CCV	Constant coefficient of variation
CLcr	Creatinine clearance (mL/min/1.73 m^2)
CL	Clearance (L/hr)
CLd	Distribution clearance (L/hr)
cov	Covariate
CABP	Community-acquired bacterial pneumonia
C24	Plasma concentration at 24 hours after a dose
CFU	Colony forming units
C_{max}	Maximum plasma concentration (mg/L)
CMT	Compartment
CV	Coefficient of variation
CWRES	Conditional weighted residuals
df	Degrees of unbounddom
dL	Deciliter(s)
ePD	Enhanced Pharmacodynamics, LLC
ETA, η	Random effect or interindividual error term for PK parameters
ε	Random effect for residual variability
ε_{ADD}	Random effect for the additive component of residual variability
ε_{CCV}	Random effect for the constant coefficient of variation component residual variability
f_{ub}	Fraction unbound
FOCEI	First-order conditional estimation method with η-ε interaction
g	Gram(s)
HABP	Hospital-acquired bacterial pneumonia
hr	Hours(s)
IBW	Ideal body weight (kg)

Abbreviation	Definition
ICU	Intensive care unit
IIV, ω^2	Interindividual variability
IOV	Interoccasion variability
IWRES	Individual weighted residuals
IV	Intravenous
k_{el}	Terminal phase elimination rate constant (hr^{-1}) determined using noncompartmental methods
kg	Kilogram(s)
L	Liter(s)
LC-MS	Liquid chromatography assay with mass spectrophotometry detection
LLOQ	Lower limit of quantification
m	Meter(s)
MDR	Multi-drug resistant
MIC	Minimum inhibitory concentration (mg/L)
mg	Milligram(s)
mL	Milliliters
MVOF	Minimum value of the NONMEM objective function
N	Number of observations/patients
NCA	Noncompartmental analysis
P	Probability
PK	Pharmacokinetic
PK-PD	Pharmacokinetic-Pharmacodynamic
Q12H	Every 12 hours
r^2	Coefficient of determination
RV, σ^2	Residual variability
SAEM	Stochastic approximation of the expectation-maximization algorithm
SD	Standard Deviation
SEM	Standard error of the mean
$T_{1/2,kel}$	Terminal phase elimination half-life (hr) determined using noncompartmental methods
$T_{1/2,\alpha}$	Alpha- or distribution phase half-life (hr)
$T_{1/2,\beta}$	Beta- or elimination phase half-life (hr)
T_{max}	Time that the maximum plasma concentration occurs (hr)
Vc	Central volume of distribution (L)
Vp	Peripheral volume of distribution (L)
Vss	Steady-state volume of distribution (L)
US FDA	United States Food and Drug Administration
yr	Year(s)

1 Introduction

Minocycline is a tetracycline derivative which has been approved in the United States (US) for oral and intravenous (IV) use since 1972. Minocycline inhibits bacterial protein synthesis through binding with the 30S subunit of the bacterial ribosome, most typically resulting in a bacteriostatic effect. Resistance to tetracyclines generally occurs through increased efflux or ribosomal protection; however, minocycline appears to evade most tetracycline resistance mechanisms, including some mechanisms expressed by multi-drug resistant (MDR) Acinetobacter that confer resistance to the other tetracyclines [1,2]. Minocycline has shown excellent *in vitro* microbiologic activity against multi-drug resistant (MDR) *Acinetobacter baumannii* [3].

A new formulation of Minocin® (minocycline) for Injection (referred to as Minocin IV in this protocol) was approved by the US Food and Drug Administration (FDA) in 2015 [4]. The new formulation is comprised of minocycline hydrochloride with magnesium sulfate to improve the solubility and stability of minocycline solutions at a more physiological pH, which enables administration of minocycline in a smaller volume of fluid. The approved indication for Minocin IV includes the treatment of infections due to susceptible strains of several important Gram-positive and Gram-negative pathogens, including *Acinetobacter* species.

Although there has been extensive clinical experience with minocycline in patients, PK studies of minocycline are relatively limited and were conducted primarily in the 1970s in healthy volunteers. Limited to no published minocycline PK data exists in ICU patients. PK studies of other antibiotics in critically ill patients have shown discrepancies between the anticipated PK parameters based on healthy volunteers or less ill patients. These differences included altered volumes of distribution, enhanced renal clearance, and altered drug metabolism, ultimately resulting in altered blood concentration time profiles.

Therefore, a study will be conducted to examine the pharmacokinetics (PK) of Minocin IV in critically-ill patients with Gram-negative infections in the Intensive Care Unit (ICU). The data obtained from this study will be used to develop a population PK model to describe the PK profile of minocycline in patients in the ICU and to examine potential patient covariate effects which may impact minocycline PK parameters. The final population PK model will also be utilized to perform Monte Carlo simulations to assess pharmacokinetic-pharmacodynamic (PK-PD) target attainment for minocycline against *A. baumannii at* the FDA approved Minocin IV dosing regimens.

2 Objectives

The objectives of this analysis are to:

- To develop a population PK model for minocycline using data collected from critically-ill adults, with illness known or suspected to be caused by infection with Gram-negative bacteria who participated in the ACUMIN study;
- To assess the ability of patient-level and clinical covariates to explain a portion of the interindividual variability in minocycline PK parameters in critically-ill adults, with illness known or suspected to be caused by infection with Gram-negative bacteria;
- To perform Monte Carlo simulations using the population PK model and recent MIC surveillance data for *A. baumannii* in order to:
    - generate a distribution of unbound minocycline exposures at the FDA approved minocycline dosing regimens in critically-ill adults, with illness known or suspected to be caused by infection with Gram-negative bacteria;
    - determine the ability of the FDA approved minocycline dosing regimens to achieve critical PK-PD targets against the range of MIC values observed with infections due to *A. baumannii*, including MDR strains among critically-ill adults, with illness known or suspected to be caused by infection with Gram-negative bacteria via simulations; and
    - assess whether dosing adjustments for the approved FDA minocycline dosing regimen are needed in critically-ill adults, with illness known or suspected to be caused by infection with Gram-negative bacteria, based upon on clinically-relevant covariate effects and the most recent MIC surveillance data.

2.1 Data

2.1.1 Study Design and Treatment Administration

The ACUMIN Study (DMID Protocol Number 16-0011) [5,6] is a Phase IV, multicenter, open-label study to evaluate the PK of minocycline in up to 50 PK evaluable ICU patients (out of the planned enrollment of 67 patients) who are already receiving antimicrobial therapy for a known or suspected Gram-negative infection. Each patient will receive a single 200 mg dose of Minocin IV infused over approximately 1 hour.

2.1.2 Pharmacokinetic Sample Collection

Blood samples (6 mL) to assess the PK of minocycline will be collected over a 48-hour period after treatment administration, preferably from the arm contralateral to the one used for IV infusion as specified in the Manual of Procedures [7]. However, given the anticipated difficulties involved with the collection of blood samples in the ICU for this patient population, either venous or arterial blood PK samples may be collected during the course of the study.

Blood samples will be collected pre-dose, immediately after infusion termination (~1 hour) and at 4, 12, 24, 36, and 48 hours after treatment administration. Plasma will be separated and stored frozen until shipped to the central laboratory for testing. Plasma PK samples will be analyzed for both unbound and total minocycline concentrations in plasma using a validated liquid chromatography assay with mass spectrophotometry detection (LC-MS).

2.1.3 Renal Replacement Therapy

Patients who are on or who may be considered for renal replacement therapy (RRT) during the study period will be allowed to participate in the study and will be included in the population PK analysis. Information pertaining to receipt of any RRT will be collected from 1-hour post start of infusion through 48 hours post start of infusion as specified in the Manual of Procedures [7].

2.2 Data Assembly and Handling

2.2.1 Data Transfer and Assembly

PK analysis dataset assembly will be performed by Enhanced Pharmacodynamics, LLC (ePD) using R software Version 3.2.2 or higher. Data will be provided to ePD by The Emmes Corporation as either SAS transport datasets or comma separated values (e.g., .CSV) ASCII files. Actual infusion start and stop dates and times, as well as the actual PK sampling dates and times, will be included in the time-ordered analysis dataset and used in all computations. Nominal PK sampling times will only be used in aggregate data

displays where appropriate to do so. Since either venous or arterial blood PK samples may be collected during the course of the study, information pertaining to the site of blood sample collection will also be included in the PK analysis dataset.

2.2.2 Definition of Pharmacokinetic Evaluable Population

The PK evaluable population will include those subjects who meet the following criteria: (1) have a baseline pre-dose PK sample collected; (2) receive the full infusion of study drug; (3) have at least three PK samples collected in the first 12 hours post-dose; (4) have at least 1 PK sample collected within 24 to 48 hours post-dose; and (5) have PK specimens which processed per the Manual of Procedures [7]. If any PK sample(s) is/are processed outside of the MOP specifications, the PK sample will still be evaluable if validation studies are performed and confirm that the deviation(s) does not result in invalid PK concentrations (total and free minocycline. Emmes will be responsible for reporting whether the PK specimens where processed per the Manual of Procedures met and this information should be included in the source data provided to ePD.

For patients who receive the full infusion of study drug but do not meet the aforementioned criteria for inclusion in the PK evaluable population, their available PK data may still be used in the population PK analysis if: (1) at least one PK specimen was processed per the Manual of Procedures, and (2) at least one such sample provided a quantifiable minocycline concentration available for analysis.

2.2.3 Data Exclusions and Handling of Missing Data

Depending upon the percentage of unbound and total plasma minocycline concentrations below the LLOQ, these observations will either be excluded from the population PK analysis or a likelihood-based approach such as the Beal M3 method will be utilized [8]. Data imputation strategies will be utilized if needed to complete a patient's dataset to account for missing covariate information. For example, in those individuals missing a baseline value for a continuous patient covariate, the median value of the covariate for the other patients in this study will be substituted for the individual missing a baseline value. Data imputation will not be performed for categorical variables and alternative approaches for covariate assessment will be required if necessary.

2.2.4 Identification and Handling of Outlier Concentrations

An outlier will be defined as an aberrant observation that substantially deviates from the rest of the observations within an individual. PK outlier concentrations will be excluded from this analysis given the potential for these observations to

negatively impact model convergence and/or the final parameter estimates. As noted in the US FDA guidance, including extreme values is not good practice with methods based on least-squares estimation or normal-theory estimation methods, as such outliers inevitably exert a disproportionate effect on the estimates [9].

Outlier detection in the analysis dataset *a priori* will be based primarily upon visual inspection of individual and pooled unbound and total plasma minocycline concentration-time data. Searching for additional outliers during the analysis will be based upon graphical exploration of individual and population conditional weighted residuals during structural PK model development. If the majority of the suspected outlier concentrations appear to occur at roughly the same time since last dose, additional attempts will be made to update the structural model to try to capture these observations. Additionally, if an entire intensively sampled individual PK profile fails to follow a reasonable pattern relative to the dosing history, the data from the entire subject may be excluded to prevent introducing bias into the analysis.

All identified outliers, which upon identification will be cross referenced to catheter data collection information, will subsequently remain in the analysis dataset but will be excluded from the population PK analysis. A dataset variable will be created called OMIT with codes used to define all observations excluded from the population PK analysis.

2.3 Population Pharmacokinetic Analysis

2.3.1 Statistical Methods for Nonlinear Mixed Effects Models

The population PK analysis will likely be conducted using NONMEM® Version 7.3 (ICON Development Solutions, Ellicott City, MD) implementing the first-order conditional estimation method with η-ε interaction (FOCEI). Alternatively, the stochastic approximation of the expectation-maximization algorithm (e.g., SAEM) may be used for comparison purposes or as the primary algorithm if the FOCEI method does not have acceptable performance. The plan will be to co-model both the unbound and total minocycline concentration-time data simultaneously.

For each model evaluated, NONMEM computes the minimum value of the objective function (MVOF), a statistic that is equivalent to minus twice the log likelihood of the data. In the case of hierarchical models, the change in the MVOF produced by the inclusion or deletion of a parameter is asymptotically distributed as chi-squared with the number of degrees of freedom (df) equal to the number of parameters added to or deleted from the model. Population PK models will minimally be assessed using the following criteria:

- Evaluation of individual and population mean PK parameter estimates for minocycline and their precision as measured by the percent standard error of the population mean estimate (%SEM) relative to historical values;
- Graphical examination of standard diagnostic and population analysis goodness-of-fit plots such as:
  - Observed versus both population and individual post-hoc predicted plasma minocycline concentrations;
  - Conditional population weighted residuals (CWRES) versus time since last dose, population predicted plasma minocycline concentrations, and potentially other independent variables; and
  - Individual weighted residuals (IWRES) versus individual post-hoc predicted plasma minocycline concentrations.
- Graphical examination of the agreement between the observed and individual post-hoc predicted plasma minocycline concentration-time data (individual observed and predicted overlays);
- Reduction in both residual variability (σ^2) and IIV (ω^2); and
- Comparison of MVOF for nested models or Akaike's Information Criterion (AIC) for non-nested models [10].

During structural population PK model development, the addition or deletion of a single fixed or random effects parameter to the structural models evaluated will be considered statistically significant if it contributes to a change in the MVOF of at least 6.635 units ($P<0.01$, 1 df) for nested models using a likelihood ratio test.

2.3.2 Exploratory Data Analysis

2.3.2.1 Data Description

Box and whisker plots will be used to explore the potential for any outliers in the observed concentration-time dataset at each of the scheduled PK sampling times. Queries will be generated to resolve potential erroneous time or concentration data point entries due to transcription or measurement errors. Individual and mean unbound and total minocycline plasma concentration-time plots will be presented on both a linear and semi-log scale will be visualized to ascertain the general structure that may best model the population PK profile.

2.3.2.2 Noncompartmental Pharmacokinetic Analysis

Noncompartmental analysis (NCA) will serve as an exploratory approach to generate initial PK parameter estimates of volume of distribution and clearance for both the unbound and total minocycline concentration-time data. The

statistical analysis software package R will be used to execute the NCA via the PKNCA package. For the NCA, concentrations below the LLOQ will be imputed as 0 if before the first measurable concentration, and treated as missing otherwise. The following PK parameters will be calculated for both the unbound and total minocycline concentrations:

- Maximum plasma minocycline concentration (C_{max}) and the time at which C_{max} occurs (T_{max});
- Plasma minocycline concentration at 24 hours after a dose (C_{24});
- Area under the plasma minocycline concentration-time curve from time zero to 24 hours (AUC_{0-24}) and to the last quantifiable sample (AUC_{0-last}) using the linear trapezoidal rule (linear up, log down calculation method);
- The terminal-phase elimination rate constant (k_{el}) obtained using linear regression (uniform weighting) and will only be estimated if there are a minimum of three concentrations available after T_{max};
- The terminal phase elimination half-life ($T_{1/2,kel}$) and
- Area under the plasma minocycline concentration-time curve from time zero to infinity ($AUC_{0-\infty}$); values of $AUC_{0-\infty}$ will be flagged in the report and excluded from calculation of summary statistics if the extrapolated area exceeds 20% of the total area.

2.3.3 Structural Population Pharmacokinetic Model Development

2.3.3.1 Structural Pharmacokinetic Model for Minocycline

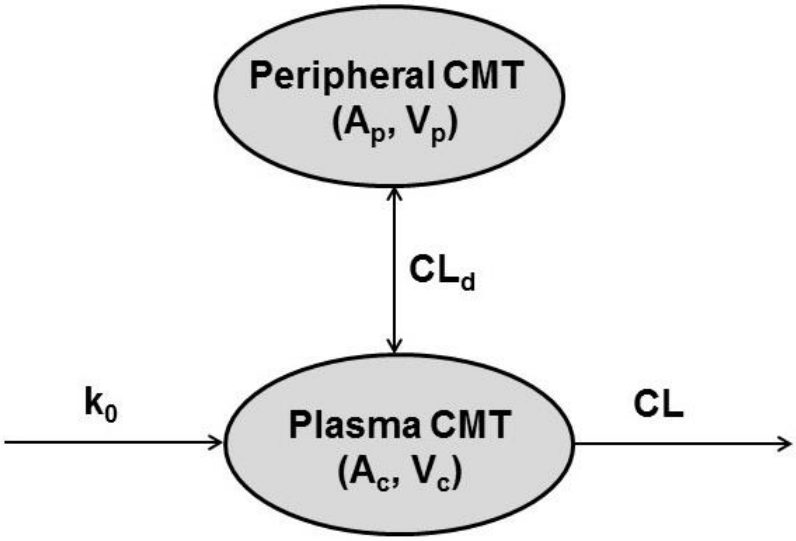
A structural compartmental PK model will be constructed to describe the time-course of unbound and total minocycline concentrations in plasma following a single IV infusion to the critically-ill patients with suspected Gram-negative infections. Plasma minocycline PK data following IV administration have previously been described using a linear two-compartment model with zero-order input and first-order elimination [11] although the assay was based upon minocycline activity and likely was not as accurate and sensitive as the LC-MS method utilized in the current study.

Therefore, structural PK model development for the ACUMIN Study data will begin with utilizing this same two-compartment model to fit the total plasma minocycline concentration-time data. Other structural models or modifications (e.g., inclusion of non-linear plasma protein binding) will be compared and contrasted as needed to identify the most parsimonious model which provide the best possible fit to the data from the critically ill patients. The two-

compartment model (**Figure 1**) will be parameterized using clearance (CL, in L/hr), central volume of distribution (Vc, in L), distributional clearance between the central compartment and the peripheral compartment (CLd, in L/hr), and the volume of distribution for the peripheral compartment (Vp, in L).

Initially, it will be assumed that plasma protein binding is constant and the fraction unbound (f_{ub}) will be estimated in order to co-model the unbound and total minocycline concentration-time data. Unbound minocycline concentrations will then be determined as the product of total minocycline concentrations and f_{ub}. If it appears that the constant plasma protein binding assumption is not adequate, saturable binding or other functions for plasma protein binding will be incorporated into the structural PK model and used to determine unbound minocycline concentrations.

Figure 1. Schematic diagram for the two-compartment structural PK model with zero-order input and first-order elimination to be fit to the total minocycline concentration-time data



2.3.3.2 Accounting for Differences in Arterial and Venous Blood Minocycline Concentrations

For a large number of compounds, studies have demonstrated different PK concentrations of a given drug molecule between simultaneously timed PK draws from arterial and venous sites [12]. This phenomenon is due to equilibration/distribution of drug in the blood and is coined the arteriovenous

concentration difference. The degree of difference is typically most pronounced during the early period post intravenous (IV) administration but can persist for hours to days. In most cases, arterial concentrations will be higher than venous concentration post IV administration of the compound. However, as the compound in arterial circulation continues to decrease through elimination and distribution into other extravascular tissues, the drug retained or extracted by the sampling tissue will begin to diffuse through the capillary wall into the venous system, resulting in venous concentration which is higher than arterial. The extent of the arteriovenous concentration difference for free and total minocycline is unknown.

Apparent differences in minocycline PK which may result from inclusion of minocycline concentrations measured in venous or arterial blood will be evaluated *a priori* as part of the base structural PK model. To accomplish this, as indicator variable designating the origin of each blood PK sample (venous or arterial) will be included in the PK analysis dataset. An empirical proportional shift in the individual predicted concentration will be evaluated for the arterial plasma minocycline concentrations as part of the structural population PK model to account from any potential differences relative to venous plasma minocycline concentrations. However, unless arterial and venous blood samples are collected within the same subject (preferably at the same PK blood draw times), it may not be possible to identify the magnitude of the arteriovenous difference as this would get masked by interindividual variability.

2.3.3.3 Interindividual Variability

Interindividual variability (IIV or ω^2) will be estimated for CL, Vc, CLd and Vp for both unbound and total minocycline plasma concentrations using exponential error models assuming these parameters are log-normally distributed as shown in **Equation 1**.

$$X_j = \tilde{X}_j \bullet e^{\eta_j^x} \quad \textbf{(1)}$$

Where:

X_j = the true value of the X parameter in the *j*th individual;

$\tilde{X}_j$ = the typical value or population mean value of the X parameter in the *j*th individual; and

η_j^x = the persistent difference between the true and the population mean value of the X parameter in the *j*th individual (referred to as ETA); the η_j^x are independent, identically distributed Gaussian random variables with a mean of 0 and a variance equal to ω^2.

This model for IIV assumes that the variance is constant with respect to the log of the population mean value of the parameter and estimates and the magnitude will be presented as percent coefficients of variation (%CV) determined as the square root of the variance multiplied by 100.

2.3.3.4 Residual Variability

Residual variability (σ^2) represents a composite of assay variability, intra-subject variability, model misspecification, errors in timing of dose and sample information, subject non-compliance, and other unexplained errors. Separate combined additive plus constant coefficient of variation (CCV) error models, as shown in **Equation 2**, will initially be used to describe residual variability for the unbound and total plasma minocycline concentrations, respectively.

$$Cp_{ij} = C\hat{p}_{ij} \bullet (1 + \varepsilon_{CCV,ij}) + \varepsilon_{ADD,ij} \quad \textbf{(2)}$$

Where:

Cp_{ij} = The measured value of the *i*th unbound or total plasma minocycline concentration (in the *j*th individual;

$C\hat{p}_{ij}$ = The *i*th unbound or total plasma minocycline concentration in the *j*th individual predicted using the specified model;

$\varepsilon_{ADD,ij}$ = Random variable which represented the additive component (e.g., intercept term) of residual variability; the $\varepsilon_{ADD,ij}$ were independent, identically distributed statistical errors with a mean of 0 and a standard deviation of σ_{ADD}; and

$\varepsilon_{CCV,ij}$ = Random variable which represented the constant coefficient of the variation or proportional component (e.g., slope term) of residual variability; the $\varepsilon_{CCV,ij}$ were independent, identically distributed statistical errors with a mean of 0 and a standard deviation of σ_{CCV}.

The additive plus CCV residual variability model allows for the variance to have a positive lower limit and then increase in proportion to the predicted unbound or total plasma minocycline concentration. Residual variability for this model will be reported as a %CV for selected values of the predicted unbound or total plasma minocycline concentrations, as shown in **Equation 3**.

$$\%CV = \frac{(\hat{Cp}_{ij} \bullet \sigma_{CCV} + \sigma_{ADD})}{\hat{Cp}_{ij}} \bullet 100\% \quad \textbf{(3)}$$

The additive plus CCV residual variability model will be reduced to only a CCV error model by fixing the additive term to zero if it can be shown that the absolute value of the individual weighted residuals, |IWRES|, are constant when examined graphically over the range of individual predicted concentrations, or if the additive component is estimated to be negligible.

2.3.4 Forward Selection of Patient Covariates

After developing a base structural population PK model to describe the unbound and total plasma minocycline concentrations, a formal covariate analysis will be initiated in NONMEM using stepwise forward selection. All parameter-covariate relationships for continuous covariates will be centered around the median value for that covariate in the ACUMIN study population. For categorical covariates, a minimum of 20% of patients need to be present in each category in order to be considered for inclusion in the covariate analysis.

Patient descriptors that will be evaluated for their potential to explain a portion of the IIV in selected unbound and total plasma minocycline PK parameters will included sex, age in years, weight in kg, height in cm, body surface area (BSA) in m^2 as calculated using the method of Gehan and George [12], body mass index (BMI) in kg/m^2, ideal body weight (IBW) in kg [14], albumin in g/dL, creatinine clearance (CLcr) in mL/min/1.73 m^2 as calculated using the Cockcroft and Gault method [15] and normalized to a BSA of 1.73 m^2 (IBW will also be substituted for total body weight for those patients whose total body weight exceeds IBW). Both baseline CLcr and potentially a time-varying CLcr may be considered in this analysis given that multiple SCr measures may be obtained after the start of therapy. Linear interpolation of SCr will be utilized to allow for a more gradual change in SCr between actual measured SCr measures. Use of RRT will also be considered as a covariate using a function to be determined based upon the type of RRT utilized, the timing of RRT relative to dosing and PK sample collection, and potentially other more detailed

information provided for each patient. If detailed information about RRT is not provided for each patient, an indicator variable will be created classifying the patient as having undergone or not undergone RRT during the study. APACHE II score and other disease related indices such as the presence of hepatic cirrhosis, heart failure, diabetes, etc. will also be evaluated as patient covariates if possible.

Using the base structural PK model, individual post-hoc PK parameters will first be obtained for each individual. Plots of these individual post-hoc parameter estimates minus the population mean value of the parameter will be examined for observable trends as an initial screening step and helped to determine which mathematical model might best describe the relationship between the PK parameter and the covariate. Mathematical models which will be considered for describing PK parameter versus continuous covariate relationships will include but will not be limited to linear (**Equation 4**) and power (**Equation 5**) functions.

Linear: $$\tilde{X}_j = \theta_X + \theta_{cov} \bullet \left(cov_j - \overline{cov}\right) \quad \textbf{(4)}$$

Power: $$\tilde{X}_j = \theta_X \bullet \left(cov_j / \overline{cov}\right)^{\theta_{cov}} \quad \textbf{(5)}$$

Where:

$\tilde{X}_j$ = the estimated typical parameter value in the *j*th individual;

cov_j = the measured value of a particular covariate in the *j*th individual;

$\overline{COV}$ = the median value of a particular covariate in the population which was utilized for centering purposes;

θ_X = the typical parameter value for individuals at the median value of a particular covariate in the population;

θ_{cov} = **linear:** the population mean estimate describing the change in the typical parameter value per unit change in the covariate;
exponential: the population mean estimate describing the change in the log of the typical parameter value per unit change in the covariate;
power: the population mean estimate describing the change in the log of the typical parameter value per unit change in the log of the covariate.

If the functional form of the parameter-continuous covariate relationship cannot be clearly elucidated from the diagnostic plots, multiple forms will be tested in NONMEM and the one that produces the most statistically significant change in the MVOF will be selected if applicable.

Dichotomous and categorical variables will be evaluated using either proportional or additive shifts as shown in **Equations 6** and **7** (in the case of categorical variables with more than two (i.e. *N*) possible levels, *N-1* dichotomous indicator variables will be created):

Proportional Shift: $$\tilde{X}_j = \theta_X \bullet \left(1 + \theta_{cov} \bullet cov_j\right) \quad \textbf{(6)}$$

Additive Shift: $$\tilde{X}_j = \theta_X + \theta_{cov} \bullet cov_j \quad \textbf{(7)}$$

Where:

$\tilde{X}_j$ = the estimated typical parameter value in the *j*th individual;

cov_j = the value of the variable (either 0 or 1) defined for a specific dichotomous covariate or indicator variable in the *j*th individual;

θ_X = the estimated population mean parameter value for individuals who belong to the reference population; and

θ_{cov} = the mean proportional or additive increase or decrease in θ_X for individuals with $cov_j = 1$.

A formal univariate analysis of each patient covariate demonstrating an observable trend with a structural PK model parameter and which is biologically plausible will then be performed in NONMEM during stepwise forward

selection. Covariates contributing at least a 6.635 change in the MVOF (α=0.01, 1 df) will be considered statistically significant during forward selection. The covariate that contributed the most significant change in the MVOF (smallest p-value <0.01) will be included in the base covariate model provided that: (1) the model converges and minimizes successfully when run in NONMEM, (2) there was a decrease in ω^2 for the parameter on which the covariate was added, and (3) there was not a substantial compensatory increase in σ^2 or ω^2 for the other PK parameters. Failure to meet all of these criteria may result in opting to not select that particular covariate effect for inclusion in the population PK model despite a statistically significant reduction in MVOF.

The new base covariate model (structural model + 1 significant covariate) will then be used to generate individual post-hoc PK parameter estimates for the next step of forward selection. During forward selection, colinearity among covariates (especially among the various body size measures, or between variables such as age and CLcr) will be considered prior to deciding which additional covariate effects will be further evaluated in NONMEM. This entire process will then be repeated until none of the remaining covariates produce a statistically significant reduction in the MVOF.

2.3.5 Full Multivariable Model Evaluation

After completion of forward selection, the IIV models will be re-evaluated. Pair-wise comparisons of the IIV terms for each parameter (ETA or η) will be graphically examined for possible correlations using a scatterplot matrix. If at least moderate correlations are observed between the η for any pairs of PK parameters (e.g., Pearson's correlation $r^2 > 0.25$), an attempt will be made to estimate the corresponding covariance (e.g., off diagonal elements of the variance-covariance matrix) between those parameters in the population PK model. In addition, the distribution of the η for each parameter will be examined for irregularities (e.g., skewness, bi-modalities, etc.) and alternative IIV models or transformations (e.g., Box-Cox) will be considered if necessary.

After making any potential adjustments to the IIV models or the variance-covariance matrix structure, the focus will shift toward correcting any potential biases or looking for ways to simplify the residual variability model. When necessary, the additive plus CCV residual error model will be simplified to a CCV error model if the data does not continue to support the estimation of the more complex model.

2.3.6 Backward Elimination of Patient Covariates

Univariate stepwise backward elimination will then be performed after all adjustments are made to the IIV and residual variability models. A patient covariate will be considered statistically significant if it contributes to at least a 10.83 increase in the MVOF (α = 0.001, 1 df) when removed from the model. During each step of backward elimination, the most non-significant covariate (the highest P-value > 0.001) will be removed from the model until all remaining covariates in the model are statistically significant.

2.3.7 Final Pharmacokinetic Model

The final population PK model will be reported as shown in **Appendix 1**, and will be assessed using all of the same model diagnostic and evaluation criteria as described previously. In addition, the overall distribution of the normalized prediction distribution errors (NPDE) provided by NONMEM for each unbound and total plasma minocycline concentration will also evaluated and compared to a normal distribution to determine if the fixed or random effects models are biased. The final NONMEM population PK model control stream, potentially shown in **Appendix 2**, along with the final output will be provided in Appendices to the final technical report.

2.3.8 Final Model Evaluation

The final population PK model will further be evaluated by performing a dose-stratified visual predictive check (VPC), which graphically examines the agreement between the 5^{th}, 50^{th}, and 95^{th} percentiles of the observed and the individual simulated unbound and total plasma minocycline concentrations across time intervals for each minocycline dose group administered [16]. The original analysis dataset will be used as a template to simulate PK data for the same number of subjects in 1,000 new datasets (NSUBPROBLEMS=1000 option for $SIMULATION within NONMEM) with each dataset featuring the same study design and data collection scheme. Based upon graphical review of these plots, the population PK model will be refined as appropriate to try to correct any substantial issues with respect to the fixed or random effects parameters in the model if there is discordance between the 5^{th}, 50^{th}, and 95^{th} percentiles of the observed and the individual simulated unbound and total plasma minocycline concentrations over time following a dose.

2.3.9 Generation of Individual Pharmacokinetic Parameters

The individual patient dosing histories will be utilized along with the individual post-hoc PK parameters to generate unbound and total plasma minocylcine concentration-time profiles and calculate unbound and total plasma minocycline exposure measures (C_{max}, C_{24}, and AUC_{0-24}). The C_{max} will be

calculated for each simulated patient as the maximum simulated concentration. The AUC_{0-24} will be calculated using numerical integration using the data from 0 to 24 hours post-dose. The $AUC_{0-\infty}$ will be calculated as Dose/CL. The steady-state volume of distribution (Vss) will be calculated as the sum of the central and peripheral volume terms. The alpha-phase half-life ($T_{1/2,\alpha}$) and beta-phase half-life ($T_{1/2,\beta}$) will also be calculated for each patient using the individual post-hoc PK parameters. Summary statistics (mean, standard deviation, median, minimum and maximum) will be calculated using the individual unbound and total CL, Vc, Vp, Vss, $T1/2_{\alpha}$ and $T1/2_{\beta}$ values. A listing of the individual PK parameters (CL, Vc, CLd, Vp, $T_{1/2,\alpha}$,$T_{1/2,\beta}$, C_{max}, C_{24}, AUC_{0-24} and $AUC_{0-\infty}$) derived using the final population PK model will be provided for each patient.

2.4 Monte Carlo Simulations

Using the final population PK model including all statistically significant covariate effects, Monte Carlo simulation will be performed using an R Shiny application (via the MRGSOLVE package) in order to generate unbound and total plasma minocycline concentration-time data after a single dose and again at steady-state in a virtual population of 2000 critically ill patients with Gram-negative infections. Minocycline doses of 100 to 200 mg will be evaluated when infused IV over 1-hour every 12 hours.

As a model checking procedure, the median and 90% prediction interval will be determined at each time for the individual post-hoc predicted unbound and total plasma minocycline concentrations for each regimen studied. Linear and semi-log plots will then be created to examine the observed data overlaid upon the median and 90% prediction interval for the individual post-hoc predicted unbound and total plasma minocycline concentrations after the first dose. The purpose of this model checking procedure is to confirm that the model simulations adequately can reproduce the observed data collected from patients in the ACUMIN study. After ensuring the model simulations were conducted appropriately, the area under the unbound plasma minocycline concentration-time curve over a 24-hour period at steady-state will then be calculated for each simulated patient using numerical integration.

The median and 90% prediction interval will also be assessed for separate simulated sub-populations (e.g, for various renal function groups) in order to evaluate the effects of clinical covariates included in the final model.

2.5 Pharmacokinetic-Pharmacodynamic Target Attainment Assessment

The PK-PD index associated with the antimicrobial efficacy of minocycline and other tetracyclines against *A. baumannii* is known to be unbound-drug

AUC:MIC ratio (*f*AUC:MIC) [17]. The minocycline *f*AUC:MIC ratio resulting in 1-log change in log_{10}CFU, which will be considered the minimum killing required for efficacy against *A. baumannii*, was recently determined using four different clinical isolates in a rat lung pneumonia infection model [18]. The minocycline *f*AUC:MIC ratios which resulted in bacterial stasis and 1-log change in log_{10}CFU was on average approximately 12 and 18, respectively, across the four different clinical isolates of *A. baumannii* studied (MICs ranged from 0.25 to 4 mg/L).

The percent of simulated patients achieving the *f*AUC:MIC target of either 12 (bacterial stasis) or 18 (1-log kill) will minimally be evaluated for the FDA approved IV mincocycline dosing regimens of 100 and 200 mg simulated and assessed over a range of MIC values for *A. baumannii.* Recent surveillance MIC data will be obtained from Melinta Therapeutics and utilized in this analysis. The PK-PD target attainment results will be presented tabularly and also graphically by overlaying the PK-PD target attainment rates upon histograms of the observed MIC distribution for *A. baumannii* as shown in the example provided in **Appendix 3**.

The PK-PD target attainment rates will also be assessed for separate simulated sub-populations (e.g., for various renal function groups) in order to evaluate the effects of clinical covariates included in the final model. Collectively, these PK-PD target attainment assessments will allow for recommendations to be made as to the adequacy of the FDA approved minocycline dosing regimens for use in critically ill patients overall and for special at-risk populations. These sub-populations simulations will also be used to determine the need for dose adjustments across clinical covariates included in the final population PK model.

3 References

1. Ritchie DJ, Garavaglia-Wilson A. A review of intravenous minocycline for treatment of multidrug-resistant Acinetobacter infections. Clin Infect Dis 2014;1.
2. Goff DA, Kaye KS. Minocycline: an old drug for a new bug: multidrug-resistant *Acinetobacter baumannii*. *Clin Infect Dis* 2014;59 Suppl 6:S374-80.
3. Bowers DR, Cao H, Zhou J, Ledesma KR, Sun D, Lomovskaya O, Tam VH. Assessment of minocycline and polymyxin B combination against *Acinetobacter baumannii*. *Antimicrob Agents Chemother*. 2015 May;59(5):2720-5.
4. Melinta Therapeutics. Minocycline for injection. Available at: www.melintamedicalinformation.com/media/1125/minocin_us_pi_may2019.pdf. Published May, 2019.
5. Division of Microbiology and Infectious Diseases (DMID). An open-label pharmacokinetic study of minocycline for injection following a single infusion in critically-ill adults (ACUMIN). DMID Protocol Number 16-0011 Version 2.0. October 31, 2017.
6. Division of Microbiology and Infectious Diseases (DMID). An open-label pharmacokinetic study of minocycline for injection following a single infusion in critically-ill adults (ACUMIN). DMID Protocol Number 16-0011 Version 3.0. November 19, 2018.
7. Division of Microbiology and Infectious Diseases (DMID). An open-label pharmacokinetic study of minocycline for injection following a single infusion in critically-ill adults (ACUMIN). Manual of Procedures for DMID Protocol Number 16-0011 Version 6.0. December 28, 2018.
8. Beal SL. Ways to fit a PK model with some data below the quantification limit. *J Pharmacokinet Pharmacodyn* 2001;28:481-504.
9. Food and Drug Administration, U.S. Department of Health and Human Services. Guidance for industry on population pharmacokinetics; availability. Food and Drug Administration, HHS. Notice. Fed Regist 1999 Feb 10; 64(27):6663-4.
10. Akaike H. Bayesian extension of the minimum AIC procedure of autoregressive model fitting. *Biometrika* 1979;66:237-242.
11. Welling PG, Shaw WR, Uman SJ, Tse FL, Craig WA. Pharmacokinetics of minocycline in renal failure. *Antimicrob Agents Chemother.* 1975; 8:532.

12. Chiou WL. The phenomenon and rationale of marked dependence of drug concentration on blood sampling site: implications in pharmacokinetics, pharmacodynamics, toxicology and therapeutics (Part I). *Clin Pharmacokinet* 1983. 17(3): 175-199.
13. Gehan EA, George SL. Estimation of human body surface area from height and weight. *Cancer Chemother Rep*. 1970;54:225-235.
14. Pai MP, Paloucek FP. The origin of the "ideal" body weight equations. *Ann Pharmacother* 2000;34:1066-1069.
15. Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. *Nephron* 1976;16:31-41.
16. Bergstrand M, Hooker AC, Wallin JE, Karlsson MO. Prediction-corrected visual predictive checks for diagnosing nonlinear mixed-effects models. *AAPS J*. 2011;13(2):143-151.
17. Agwuh KN, MacGowan A. Pharmacokinetics and pharmacodynamics of the tetracyclines, including glycylcyclines. *J Antimicrob Chemother.* 2006; 58, 256-265.
18. Tatrzi Z, Sabet M., Dudley MN, Griffith DC. Pharmacodynamics of minocycline against *Acenitobacter baumannii* in a rat pneumonia model. Abstract submitted to 55th ICAAC, 2015, San Diego, CA.

4 Appendices

Appendix 1. Example of potential final population PK model parameter estimate table

Table X. Parameter estimates and associated standard errors for the final population PK model for total and unbound minocycline

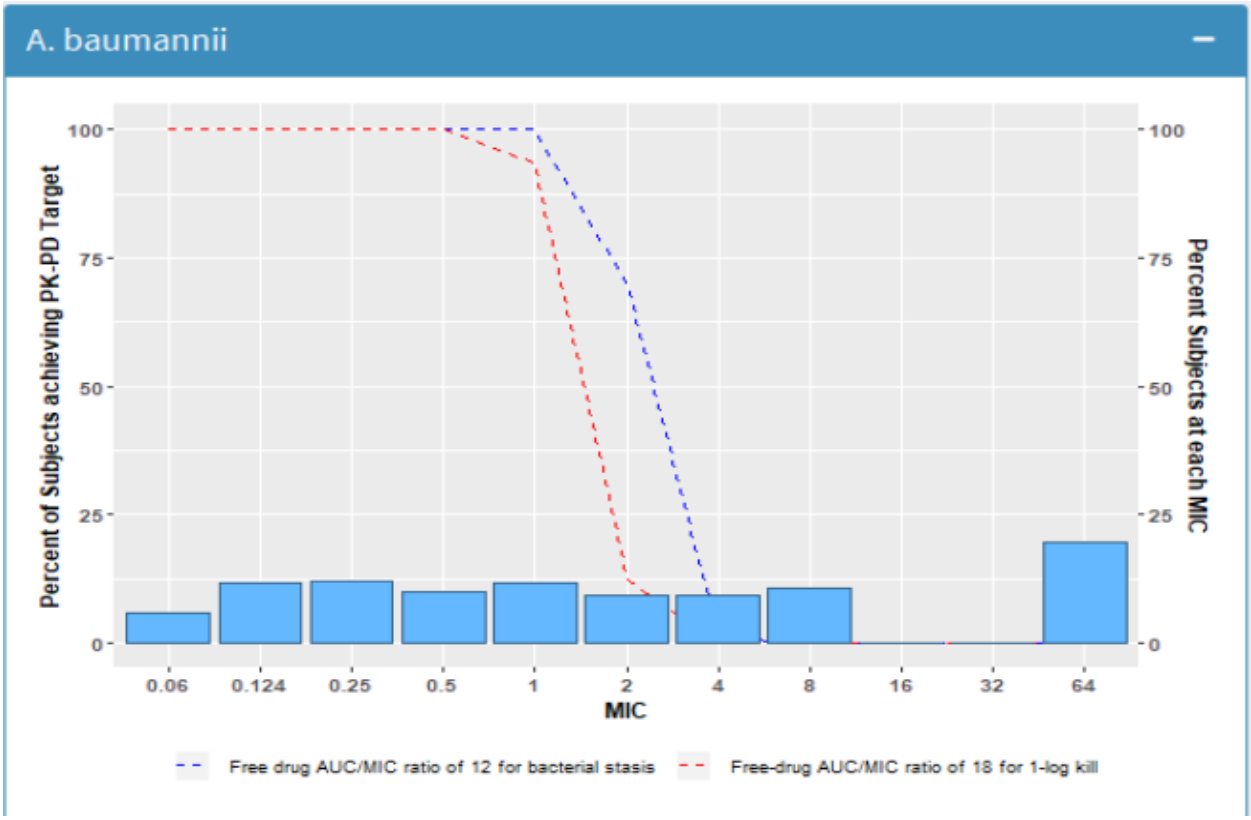
Parameter	Final estimate	%SEM
f_{ub}	X	X
CL (L/hr)	X	X
Vc (L)	X	X
CLd (L/hr)	X	X
Vp (L)	X	X
ω^2 for CL	X (X%CV)	X
ω^2 for Vc	X (X%CV)	X
ω^2 for CLd	X (X%CV)	X
ω^2 for Vp	X (X%CV)	X
Residual variability (σ^2)		
Total	X (X%CV)	X
Unbound	X (X% CV)	X

Appendix 2. Example NONMEM control stream for the final population PK model for total and unbound plasma minocycline concentrations

```
$PROB Population PK Model for total and unbound minocycline

$INPUT OMIT ID etc....

$DATA ..\..\..\datasets\pk\allpk.csv
    IGNORE=O
    IGNORE=(OMIT.GE.1)

$SUBROUTINES ADVAN13 TRANS1 TOL=5 SUBROUTINES=D

$MODEL COMP=(TCENTRAL,DEFDOSE,DEFOBS)  ; Total Central CMT
       COMP=(TPERIPH,NODOSE)           ; Total Peripheral CMT
       COMP=(CENTRAL,NODOSE)           ; Unbound drug

$PK

  CALLFL=-2

  TVCL=THETA(1)*(CLCRN/85)**THETA(5)
  CL=TVCL*EXP(ETA(1))

  TVVC=THETA(2)
  VC=TVVC*EXP(ETA(2))

  TVCLD=THETA(3)
  CLD=TVCLD*EXP(ETA(3))

  TVVP=THETA(4)
  VP=TVVP*EXP(ETA(4))

  FUB=THETA(6)

  K12=CLD/VC
  K21=CLD/VP
  K10=CL/VC

  S1=VC     ;dose in mg, conc in mg/L

$DES

  DADT(1) = K21*A(2) - K12*A(1)  - K10*A(1)      ; Total Central CMT
  DADT(2) = K12*A(1) - K21*A(2)                  ; Total peripheral CMT

$ERROR (ONLY OBSERVATIONS)

IF(CMT.EQ.1)THEN
   IPRED=A(1)/S1
   IRES=DV-IPRED
   Y=IPRED + IPRED*EPS(1) + EPS(2)
   W=SQRT(IPRED**2*SIGMA(1,1)+SIGMA(2,2))
   IWRES=IRES/W
ENDIF
```

```
IF(CMT.EQ.3)THEN
   IPRED=(A(1)/S1)*FUB
   IRES=DV-IPRED
   Y=IPRED + IPRED*EPS(3) + EPS(4)
   W=SQRT(IPRED**2*SIGMA(3,3)+SIGMA(4,4))
   IWRES=IRES/W
ENDIF

;initial estimates for thetas
                          ; parm          units
$THETA (0,2.33)           ; CL            L/hr
       (0,9.5)            ; VC            L
       (0,0.985)          ; CLD           L/hr
       (0,100)            ; VP            L
       (0,0.56)           ; CLCRN_CL      none
       (0,0.85,1)         ; FUB           none

;initial estimates for etas
                          ; parm          units
$OMEGA BLOCK(1) 0.115     ; IIV_CL        %CV
$OMEGA BLOCK(1) 0.0488    ; IIV_VC        %CV
$OMEGA BLOCK(1) 0.112     ; IIV_CLD       %CV
$OMEGA BLOCK(1) 0.0885    ; IIV_VP        %CV

;initial estimates for epsilons
                          ; parm       units
$SIGMA 0.0427             ; RV_CCV_TOT %CV
       0 FIXED            ; RV_ADD_TOT SD
       0.0427             ; RV_CCV_UB  %CV
       0 FIXED            ; RV_ADD_UB  SD


$EST METHOD=CONDITIONAL INTERACTION MAXEVAL=9999 PRINT=5 NOABORT
     NOTHETABOUNDTEST NOOMEGABOUNDTEST NOSIGMABOUNDTEST NSIG=2 SIGL=6

$COV UNCONDITIONAL

$TABLE ID TIME AMT CMT TSLD DOSEMG ETA1 ETA2 ETA3 ETA4 TVCL TVVC TVCLD TVVP FUB
       CL VC CLD VP IPRED CWRES IRES IWRES NOPRINT ONEHEADER FILE=base-1.tbl
```

Appendix 3. Example of plotting the PK-PD target attainment results over the recent observed MIC distribution surveillance data for *A. baumannii*



PK-PD Target Statistics

MIC	Percent of Subjects Achieving Free-drug AUC/MIC ratio of 12 for A. baumannii (net bacterial stasis)	Percent of Subjects Achieving Free-drug AUC/MIC ratio of 18 for A. baumannii (1-log10 CFU kill)
0.06	100	100
0.124	100	100
0.25	100	100
0.5	100	100
1	100	93.6
2	69.7	12.5
4	0.9	0
8	0	0
16	0	0
32	0	0
64	0	0