A Phase IV Open-Label Pharmacokinetic Study of Minocycline for Injection Following a Single Infusion in Critically-Ill Adults (ACUMIN)

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IND Sponsor: Division of Microbiology and Infectious Diseases (DMID)

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Version Number: 3.0

19 November, 2018

STATEMENT OF COMPLIANCE

The study will be carried out in accordance with Good Clinical Practice (GCP) as required by the following *(use applicable regulations depending on study location and sponsor requirements; samples follow)*:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46; 21 CFR Part 50, 21 CFR Part 56, and 21 CFR Part 312)
- ICH E6; 62 Federal Register 25691 (1997)
- Clinical Terms of Award

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

SIGNATURE PAGE

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

Site Investigator:

Signed: _____ Date: _____

Name

Title

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LIST OF ABBREVIATIONS

AE	Adverse Event
Alpha, α	Level of significance or distribution-phase elimination rate constant
ARLG	Antibacterial Resistance Leadership Group
ALT	Alanine aminotransferase
ARLG	Antibacterial Resistance Leadership Group
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
BLQ	Below Limit of Quantitation
BPM	Beats per minute
BUN	Blood urea nitrogen
CFR	Code of Federal Regulations
CI	Confidence interval
CL	Clearance
CLd	Distribution clearance
Cmax	Maximum plasma drug concentration
C_{24}	Plasma drug concentration at 24-hours after a dose
CROMS	Clinical Research Operational Management Support
DHHS	Department of Health and Human Services
DMID	Division of Microbiology and Infectious Diseases, NIAID, NIH, DHHS
eCRF	Electronic Case Report Form

*f*AUC:MIC	Free-drug AUC:MIC ratio
FDA	Food and Drug Administration
FOCEI	First-order conditional estimation method with interaction
FWA	Federalwide Assurance
GCP	Good Clinical Practice
hCG	Human Chorionic Gonadotropin
HCl	Hydrogen chloride
ICF	Informed Consent Form
ICH	International Council for Harmonization
ICMJE	International Committee of Medical Journal Editors
ICU	Intensive Care Unit
IDES	Internet Data Entry System
IH	Intracranial hypertension
IND	Investigational New Drug Application
IRB	Institutional Review Board
IV	Intravenous
LAR	Legally Authorized Representative
LCE	Leukocyte esterase
LC-MS	Liquid chromatography assay with mass spectrophotometry detection
LLOQ	Lower Limit of Quantification
MDR	Multiple Drug Resistant
MIC	Minimum Inhibitory Concentration
mg	Milligrams
mL	Milliliters
MOP	Manual of Procedures
NCA	Non-compartmental analysis
NDA	New Drug Application

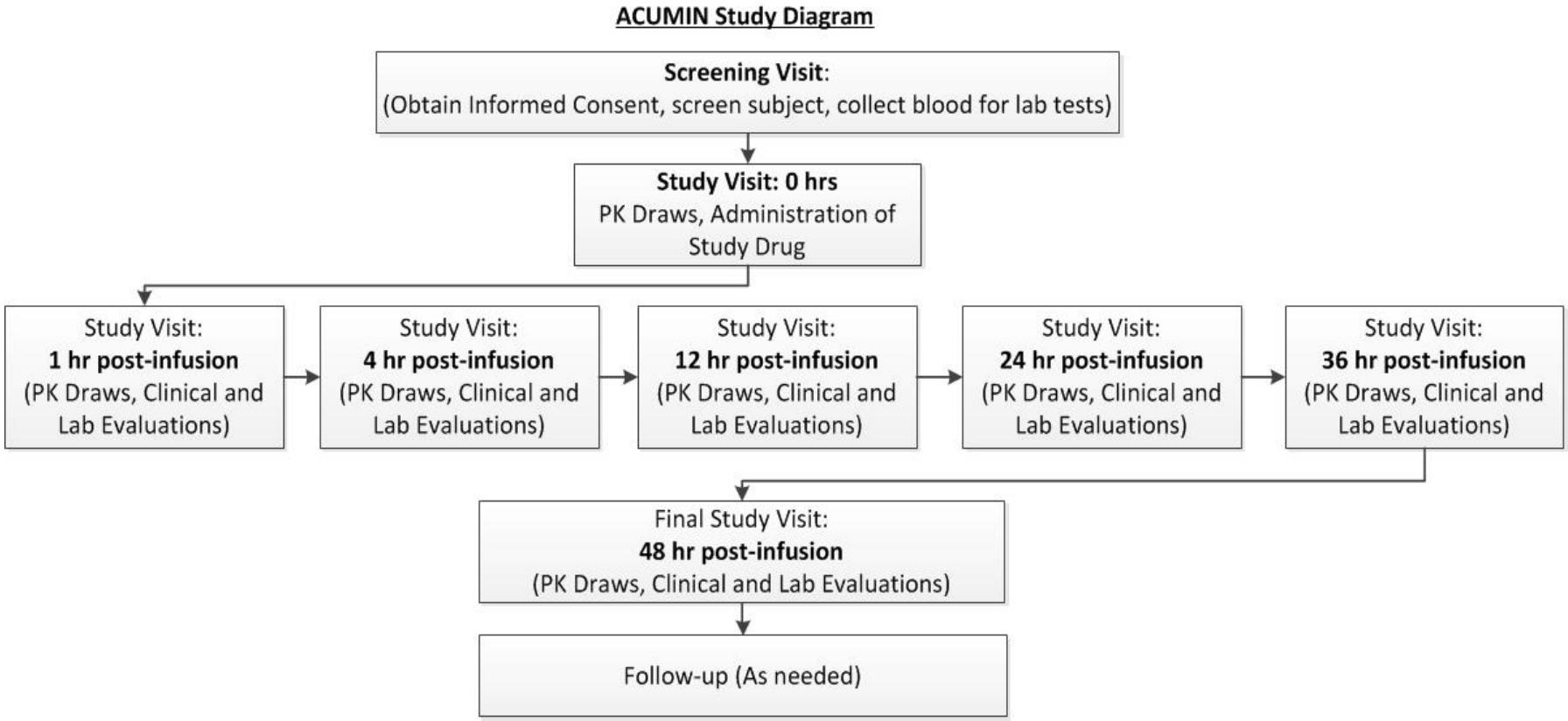
NIAID	National Institute of Allergy and Infectious Diseases, NIH, DHHS
NIH	National Institutes of Health
PD	Pharmacodynamics
PI	Principal Investigator
PK	Pharmacokinetics
QA	Quality Assurance
QC	Quality Control
RRT	Renal Replacement Therapy
SAE	Serious Adverse Event
SAEM	Stochastic approximation of the expectation-maximization algorithm
SAP	Statistical Analysis Plan
SDCC	Statistical and Data Coordinating Center
SEM	Standard error of the mean
SMC	Safety Monitoring Committee
SSR	Safety Summary Report
SUSAR	Suspected Unexpected Serious Adverse Reaction
TEAE	Treatment-Emergent Adverse Events
T_{max}	Time at which the maximum plasma drug concentration occurs
US	United States
USP	United States Pharmacopeia
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 count
WFI	Water for injection

PROTOCOL SUMMARY

Title:	A Phase IV Open-Label Pharmacokinetic Study of Minocycline for Injection Following a Single Infusion in Critically-Ill Adults (ACUMIN)
Phase:	Phase IV
Population:	Up to 67 subjects enrolled to obtain 50 evaluable, critically-ill adults with illness known or suspected to be caused by infection with Gram-negative bacteria
Number of Sites:	Approximately 13 clinical sites
Study Duration:	Approximately 16 months
Subject Participation Duration:	Approximately 2 days
Description of Agent or Intervention:	Minocin® IV (minocycline hydrochloride injection) 200mg administered intravenously as a single infusion over approximately 60 minutes
Objectives:	**Primary:** 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:** 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
	To evaluate changes of physiological parameters following a single infusion of minocycline among critically-ill adults.
Description of Study Design:	Unblinded, single-dose, population PK study
Estimated Time to Complete Enrollment:	Approximately 16 months

Figure 1: ACUMIN Study Diagram



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2 BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 Background Information

Minocycline is a tetracycline derivative first approved in the United States (US) as both oral and intravenous (IV) formulations in the 1970s. The original IV formulation of minocycline was approved in the US on 26 October 1972. Rempex Pharmaceuticals, Inc., a wholly owned subsidiary of Melinta Therapeutics, Inc., has developed a new formulation, Minocin® (minocycline) for Injection (referred to as Minocin IV in this protocol), which was approved by the US Food and Drug Administration (FDA) on 17 April, 2015 (NDA 50,444). 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. Minocycline has excellent *in vitro* microbiologic activity against multi-drug resistant *Acinetobacter baumannii* species,[1-3] a growing world-wide problem.[4-9] Furthermore, IV minocycline has been in use for over four decades and has a well-established safety profile.

2.2 Rationale

This study will examine the pharmacokinetics (PK) of Minocin IV in critically-ill patients with Gram-negative infections in the Intensive Care Unit (ICU). 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.[10]

More importantly, 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. This study will therefore develop a population PK model to describe the PK profile of minocycline in patients in the ICU. As part of

this investigation, we will investigate if patient level and clinical covariates affect the observed minocycline PK profile in patients in the ICU.

2.3 Potential Risks and Benefits

2.3.1 Potential Risks

In general, there is minimal risk of significant adverse events related to the infusion of Minocin IV. The risk of Minocin IV is consistent with taking an intravenous dose of other tetracycline antibiotic. A single dose of minocycline is unlikely to select for tetracycline resistance.

Because of the extensive and long standing use of tetracycline antibiotics there have been many reports of potential drug reactions.

The following adverse reactions have been observed in patients receiving any tetracyclines and are included in the Minocin® (minocycline) for Injection Package Insert.

Body as a whole: Fever and discoloration of secretions.

Gastrointestinal: Anorexia, nausea, vomiting, diarrhea, dyspepsia, stomatitis, glossitis, dysphagia, enamel hypoplasia, enterocolitis, pseudomembranous colitis, pancreatitis, and inflammatory lesions (with monilial overgrowth) in the oral and anogenital regions. These reactions have been caused by both oral and parenteral administration of tetracyclines.

Genitourinary: Vulvovaginitis.

Hepatic toxicity: Hyperbilirubinemia, hepatic cholestasis, increases in liver enzymes, fatal hepatic failure, and jaundice. Hepatitis, including autoimmune hepatitis and liver failure, has been reported.

Skin: Alopecia, erythema nodosum, hyperpigmentation of nails, pruritus, toxic epidermal necrolysis and vasculitis, and maculopapular and erythematous rashes. Exfoliative dermatitis has been reported. Fixed drug eruptions have been reported. Lesions occurring on the glans penis have caused balanitis. Erythema multiforme and Stevens-Johnson syndrome have been reported. Photosensitivity and pigmentation of the skin and mucous membranes have also been reported.

Local reactions: Injection site erythema and injection site pain.

Respiratory: Cough, dyspnea, bronchospasm, exacerbation of asthma, and pneumonitis.

Renal toxicity: Interstitial nephritis. Elevations in blood urea nitrogen (BUN) have been reported and are apparently dose-related. Acute renal failure has been reported.

Musculoskeletal: Arthralgia, arthritis, bone discoloration, myalgia, joint stiffness, and joint swelling.

Hypersensitivity reactions: Urticaria, angioneurotic edema, polyarthralgia, anaphylaxis/anaphylactoid reaction (including shock and fatalities), anaphylactoid purpura, myocarditis, pericarditis, exacerbation of systemic lupus erythematosus, and pulmonary infiltrates with eosinophilia have been reported. A lupus-like syndrome and serum sickness-like reactions have also been reported.

Blood: Agranulocytosis, hemolytic anemia, thrombocytopenia, leukopenia, neutropenia, pancytopenia, and eosinophilia have been reported.

Central nervous system: Convulsions, dizziness, hypoesthesia, paresthesia, sedation, and vertigo. Pseudotumor cerebri (benign intracranial hypertension [IH]) in adults and bulging fontanels in infants have been reported. Headache has also been reported.

Other: Thyroid cancer has been reported in the post marketing setting in association with minocycline products. When minocycline therapy is given over prolonged periods, monitoring for signs of thyroid cancer should be considered. When given over prolonged periods, tetracyclines have been reported to produce brown-black microscopic discoloration of the thyroid gland. Cases of abnormal thyroid function have been reported.

Tooth discoloration in pediatric patients less than eight years of age and in adults has been reported.

Oral cavity discoloration (including tongue, lip, and gum) has been reported. Tinnitus and decreased hearing have been reported.

The following syndromes have been reported; in some cases involving these syndromes, death has been reported. As with other serious adverse reactions, if any of these syndromes are recognized, the drug should be discontinued immediately:

Hypersensitivity syndrome consisting of cutaneous reaction (such as rash or exfoliative dermatitis), eosinophilia, and one or more of the following: hepatitis, pneumonitis, nephritis, myocarditis, and pericarditis. Fever and lymphadenopathy may be present.

Lupus-like syndrome consisting of positive antinuclear antibody; arthralgia, arthritis, joint stiffness, or joint swelling; and one or more of the following: fever, myalgia, hepatitis, rash, and vasculitis.

Serum sickness-like syndrome consisting of fever, urticarial, or rash; and arthralgia, arthritis, joint stiffness, or joint swelling. Eosinophilia may be present.

Minocin IV contains subtherapeutic levels of magnesium sulfate heptahydrate. Adverse effects that may be associated with magnesium intoxication include flushing, sweating, hypotension, depressed reflexes, flaccid paralysis, hypothermia, circulatory collapse, cardiac and central nervous system depression proceeding to respiratory paralysis.

2.3.2 Known Potential Benefits

This is a non-treatment study and subjects are not expected to benefit by receiving the study drug. The study will provide critical PK and PK/PD data in an ICU patient population where Minocin IV is likely to be used for the treatment of *Acinetobacter* infections.

3 OBJECTIVES

3.1 Study 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.

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

3.2 Study Outcome Measures

3.2.1 Primary Outcome Measures

Population mean PK parameter estimates and the magnitude of the associated inter-individual variability for free-drug and total-drug clearance (CL), central volume of distribution (Vc), distribution clearance (CLd), and the peripheral volume of distribution (Vp), determined using nonlinear mixed effects modeling on PK time points collected up to 48 hours after a single-dose on Day 1.

Individual post-hoc PK parameter estimates and calculated exposure measures after a single dose such as the maximum plasma concentration (C_{max}), the plasma concentration at 24 hours after a dose (C_{24}), the area under the curve from 0 to 24 hours after a dose (AUC_{0-24}), the area under the curve to the last quantifiable sample (AUC_{0-last}), and area under the curve from 0 to infinity (AUC_{0-inf}) using both free-drug and total-drug concentrations.

3.2.2 Exploratory Outcome Measures

Distribution of simulated concentration-time profiles with the FDA approved minocycline dosing regimen across the patient-level and clinical covariates patterns in the final population PK model.

Comparison of the observed simulated concentration-time profiles in critically-ill adults, with illness known or suspected to be caused by infection with Gram-negative bacteria with simulated concentration-time profiles in healthy adults. If needed, dose adjustment calculations will be made.

Probability of achieving free-drug AUC:MIC ratio (*f*AUC:MIC) ≥ 12 (PK-PD target for bacterial stasis), and probability of achieving *f*AUC:MIC ratio ≥ 18 (PK-PD target for 1-$\log_{10}$ CFU kill), for FDA-approved minocycline dosing regimens.

Change from baseline at 48hr post-dose in:

liver function tests

magnesium

serum creatinine

4 STUDY DESIGN

This is a Phase IV, multi-center open-label pharmacokinetic trial studying the pharmacokinetics and pharmacodynamics of a single dose of Minocin IV in up to 50 evaluable, ICU patients who are 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 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.

Blood specimens will be processed and shipped on dry ice to a central laboratory where total and free minocycline levels will be analyzed. Data will be reported back to Emmes, and will be shared with Albany College of Pharmacy and Emmes for PK modeling. The data will be analyzed using a population pharmacokinetic approach with covariate model building.

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.

For subjects who receive the full infusion of study drug but are not PK evaluable, their available PK data may still be used in the final PK analyses if certain criteria listed in the Statistical Analysis Plan are met.

5 STUDY ENROLLMENT AND WITHDRAWAL

5.1 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

5.2 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
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]

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

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

[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.

5.3 Treatment Assignment Procedures

5.3.1 Randomization Procedures

This is a single arm study. There are no randomization procedures. Subjects will be enrolled online through the enrollment module of AdvantagEDC[SM], the Emmes Corporation's Internet Data Entry System (IDES). Instructions for use of the enrollment module are included in the AdvantagEDC[SM] User's Guide. Manual back-up enrollment procedures are provided in the MOP for use in the event that the site temporarily loses access to the internet or the online enrollment system is unavailable.

5.3.2 Masking Procedures

This is an unblinded study. Masking is not needed.

5.3.3 Reasons for Study Withdrawal

All subjects have the right to withdraw at any point during treatment without prejudice. A subject may withdraw or be withdrawn from this study for any of the following reasons:

- Medical disease or condition, or any new clinical findings for which continued participation, in the opinion of the site principal investigator or appropriate sub-investigator, would compromise the safety of the subject, or would interfere with the subject's successful completion of the study, or would interfere with the evaluation of responses.

- As deemed necessary by the site principal investigator or appropriate sub-investigator for noncompliance or other reasons.

- Subject withdrew consent.

The investigator can discontinue any subject's Minocin infusion at any time if medically necessary. The reason(s) for early discontinuation should be reflected in the source documentation and on the appropriate eCRF.

5.3.4 Handling of Withdrawals from the PK Analysis

In all cases, the reasons why a subject is withdrawn must be recorded into the eCRF. Subjects who are not evaluable for the PK analysis will be replaced. Refer to Section 4 for the definition of an evaluable subject.

5.3.5 Termination of Study

The DMID/NIAID, ARLG, FDA and/or Rempex Pharmaceuticals, Inc./Melinta Therapeutics Inc. have the right to discontinue this study at any time for medical and/or administrative reasons. For subjects who received minocycline prior to the termination of the study, all safety related procedures described in this protocol should be performed.

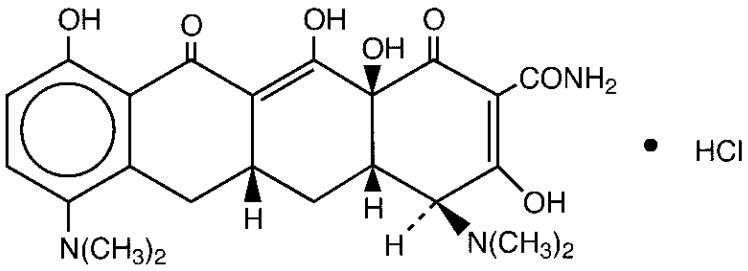
6 STUDY INTERVENTION/INVESTIGATIONAL PRODUCT

6.1 Study Product Description

Minocin IV is a sterile formulation of a semisynthetic derivative of tetracycline. The chemical name of minocycline is 4,7-Bis(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,10,12,12a-tetrahydroxy-1,11-dioxo-2-naphthacenecarboxamide monohydrochloride.

Its structural formula is:

Figure 2: Structural Formula of Minocin IV



$C_{23}H_{27}N_3O_7$•HCl M.W. 493.94

6.1.1 Acquisition

Minocin IV will be supplied by Rempex Pharmaceuticals, Inc., a wholly owned subsidiary of Melinta Therapeutics, Inc.

Sterile water for injection, USP and 0.9% Sodium Chloride, USP (normal saline) will be supplied by each participating site.

Study product will be shipped to the investigational site upon request and approval by DMID.

6.1.2 Formulation, Packaging, and Labeling

6.1.2.1 Minocin IV

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 Manual of Procedures and Minocin® (minocycline) for Injection Package Insert.

6.1.2.2 Sterile Water for Injection (WFI), USP

The sterile water for injection (WFI), USP is nonpyrogenic and contains no bacteriostatic, antimicrobial agent, or added buffer. This product should be used to reconstitute the Minocin IV vial. WFI will be obtained as a single-dose container.

6.1.2.3 0.9% Sodium Chloride, USP (Normal Saline)

0.9% Sodium Chloride Injection, USP is a sterile, nonpyrogenic, isotonic solution of sodium chloride and water for injection. Each mL contains 9 mg of sodium chloride and contains no preservatives, bacteriostatic, antimicrobial agent, or added buffer. The solution is clear in appearance and may contain hydrochloric acid and/or sodium hydroxide for pH adjustment (pH 5.3 [4.5 to 7.0]). Normal saline will be obtained in 100 mL IV bags and will be used to dilute the Minocin IV for intravenous infusion.

6.1.3 Product Storage and Stability

6.1.3.1 Minocin IV

Vials containing lyophilized Minocin IV are stored at controlled room temperature of 20°C to 25°C (68°F to 77°F). Per the USP controlled room temperature requirement, excursions between 15°C and 30°C (59°F and 86°F) are permitted. Once reconstituted, Minocin IV bags can be stored at room temperature for up to 4 hours or refrigerated at 2°C to 8°C (36°F to 46°F) for up to 24 hours. Further details can be found in the Manual of Procedures and Minocin® (minocycline) for Injection Package Insert.

6.1.3.2 Sterile Water for Injection (WFI), USP

The sterile WFI vials are stored at 20ºC to 25ºC (68°F to 77ºF) [See USP Controlled Room Temperature; excursions between 15°C and 30°C (59°F and 86°F) are permitted].

6.1.3.3 0.9% Sodium Chloride, USP (Normal Saline)

Store at 20ºC to 25ºC (68°F to 77ºF) [See USP Controlled Room Temperature; excursions between 15°C and 30°C (59°F and 86°F) are permitted]. Protect from freezing.

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

Two-100 mg vials of Minocin IV are required for each single dose. The lyophilized powder in each vial (approximately 108 mg/vial, equivalent to 100 mg upon reconstitution) should be reconstituted with 5 mL of Sterile Water for Injection (10mL total). 10 mL should be removed from the 100 mL bag of Sodium Chloride Injection, USP. The contents of the two reconstituted vials of Minocin IV should be transferred aseptically to the bag of Sodium Chloride Injection

USP, and then mixed by inversion. Once diluted into an intravenous bag, the infusion may be stored at room temperature (20° to 25°C) and administered within 4 hours, or refrigerated at 2 to 8°C (36 to 46°F) and administered within 24 hours of preparation.

Subjects will receive a single 200 mg intravenous infusion of Minocin IV in 100 mL of normal saline administered at a rate of 100 mL/hour and until the bag is empty and the line is flushed. Additional study product preparation details are further described in the protocol-specific Manual of Procedures.

6.3 Modification of Study Intervention/Investigational Product for a Subject

This is a single dose study. There will be no dose modifications permitted.

6.4 Accountability Procedures for the Study Intervention/Investigational Product(s)

The site principal investigator is responsible for the distribution and disposition of study product, and has ultimate responsibility for accountability. The site principal investigator may delegate this responsibility to the site Research Pharmacist. If delegated, the site Research Pharmacist will be responsible for maintaining complete records and documentation of study product receipt, accountability, dispensation, temperature monitoring, and storage conditions, and final disposition of the study product.

All study products, whether administered or not, must be documented on the appropriate study product accountability record or dispensing log. Used and unused Minocin IV vials will be retained until monitored and then released for disposition. Any unused solution left in the IV infusion bag or IV administration tubing after administration to the subject should be discarded as biohazardous waste.

Upon completion of the study and after the final monitoring visit, any remaining unused study product will either be returned or destroyed appropriately at the clinical site as per sponsor requirements and instructions, or in accordance with disposition plans.

6.5 Assessment of Subject Compliance with Study Intervention/Investigational Product

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 captured in the source documents.

6.6 Concomitant Medications/Treatments

In this study, a single dose of Minocin IV is being administered, and as such, is unlikely to result in significant medication interaction. However, the following are listed in the Minocin® (minocycline) for Injection Package Insert as precautions or warnings:

Because tetracyclines have been shown to depress plasma prothrombin activity, patients who are on anticoagulant therapy may require downward adjustment of their anticoagulant dosage.

Since bacteriostatic drugs may interfere with the bactericidal action of penicillin, it is advisable to avoid giving tetracyclines in conjunction with penicillin.

The concurrent use of tetracyclines and methoxyflurane has been reported to result in fatal renal toxicity.

Concurrent use of tetracyclines with oral contraceptives may render oral contraceptives less effective.

Administration of isotretinoin should be avoided shortly before, during, and shortly after minocycline therapy. Each drug alone has been associated with pseudotumor cerebri.

Increased risk of ergotism when ergot alkaloids or their derivatives are given with tetracyclines.

Minocin IV contains magnesium sulfate heptahydrate. Potentially serious drug interactions may occur when intravenous magnesium sulfate heptahydrate is given concomitantly with CNS depressants, neuromuscular blocking agents and cardiac glycosides.

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 renal replacement therapies (RRT) will be collected from 1 hour post start of infusion through 48 hours post start of infusion.

7 STUDY SCHEDULE

The following sections describe the study procedures and data collected. For each procedure, subjects are to be assessed by the investigator or site personnel. A Schedule of Events is located in Appendix A.

7.1 Screening (within 48 hours of enrollment)

Informed consent must be obtained prior to the subject entering into the study, and before any protocol-directed procedures are performed. Subjects will be screened in accordance with predefined inclusion and exclusion criteria as described in Section 5.

Subjects/LARs will be provided with a description of the study (purpose and study procedures) and asked to read and sign the informed consent form. After the subject has provided informed consent to participate in the study, screening will include:

Review eligibility criteria with the subject and/or LAR

Complete medical history

Demographic information (age, gender, race, ethnicity)

Height (can be pulled from medical records associated with the current hospital admission OR collected within the screening window)

Targeted physical examination

Vital signs

Collect concomitant medications (starting 24 hours prior to start of infusion)

Clinical laboratory evaluations to include (see Section 8.2.1 for complete list of tests)*:

- Blood chemistry
- Serum creatinine (if not included in clinical chemistry)
- Liver function tests
- Hematology
- Magnesium
- Urinalysis
- Serum hCG pregnancy test, from women of childbearing potential

* Standard of care results obtained for clinical care purposes within 48hrs of screening are acceptable for screening. A pregnancy test at any time during the current hospitalization is acceptable for screening.

7.2 Baseline (Dosing – 0 hrs)

If the subject has satisfied all of the inclusion criteria and none of the exclusion criteria, the subject should be enrolled as described in Section 5.3. The following procedures will be completed:

Blood chemistry (if not collected within 8 hours prior to the start of study drug infusion)

Liver function tests (if not collected within 8 hours prior to the start of study drug infusion)

Hematology (if not 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

Weight, collected as close to the start of the study drug infusion as possible

PK blood samples, collected just prior to the start of study drug infusion

Serum creatinine, collected just prior to the start of study drug infusion

Magnesium, collected just prior to the start of study drug infusion

Administration of a single dose of Minocin IV in the ICU under the supervision of the investigator or designee

Collection of concomitant medications, including IV medications administered during the time of Minocin infusion (refer to the Manual of Procedures for details)

Serious adverse event collection (SAEs will be collected from the start of the investigational drug infusion and will continue for 24 hours from that point.)

7.3 1-24 hour Study Visits (post start of infusion)

Vital signs collected at 1 hour and at 24 hours

Weight collected at 24 hour*s*

Collection of concomitant medications, including IV medications administered (refer to the Manual of Procedures for details)

Collection of any RRTs being received

Serious adverse event collection (SAEs will be collected from the start of the investigational drug infusion and will continue for 24 hours from that point.)

PK blood samples collected at the following time points: Immediately after the end of infusion (1 hour), 4 hours, 12 hours and 24 hours

Serum creatinine collected at the following time points: Immediately after the end of infusion (1 hour), 4 hours, 12 hours and 24 hour*s*

Magnesium collected at 24 hours

Hematology at 24 hours

NOTE: Study time point windows are detailed in the Schedule of Events (Appendix A)

7.4 36 hour Study Visit (post start of infusion)

Subjects will undergo the following procedures:

Collection of concomitant medications, including IV medications administered (refer to the Manual of Procedures for details)

PK blood samples collected at 36 hours

Serum creatinine collected at 36 hours

Collection of any RRTs being received

Follow-up on any reported SAEs

NOTE: Study time point windows are detailed in the Schedule of Events (Appendix A)

7.5 48 hour Time Point (Final Study Visit)

The final study visit will occur at 48 hours after the start of infusion. Subjects will undergo the following procedures:

Vital signs collected at 48 hours

Hematology at 48 hours

Weight

Collection of concomitant medications, including IV medications (refer to the Manual of Procedures for details)

Collection of any RRTs being received

Follow-up on any reported SAEs

PK blood samples collected at 48 hours

Clinical laboratory evaluations to include:

Liver function tests

Serum creatinine

Magnesium

NOTE: Study time point windows are detailed in the Schedule of Events (Appendix A)

7.6 SAE Follow-up Visit (as needed)

Targeted physical examination

Vital signs

Collect concomitant medications

Clinical laboratory evaluations, as needed

Follow-up on any SAEs

7.7 Early Termination Visit

If a subject is terminated early, the reason for termination must be documented in the source document and eCRF. If at all possible, vital signs and laboratory tests obtained for clinical purposes should be recorded at early termination.

7.8 Unscheduled Visits

Unscheduled visits will occur at the investigators discretion.

8 STUDY PROCEDURES/EVALUATIONS

8.1 Clinical Evaluations

The study protocol must be strictly adhered to. Any modifications that become necessary or desirable must be documented in writing. Amendments or changes to the protocol require the approval of the investigator and the Institution Review Board (IRB). Any protocol deviations will be documented.

Complete medical history will be obtained by interview of the subject or subject's LAR during screening. Subject will be queried regarding a history of significant medical disorders and any allergies.

RRT data will be collected for subjects who undergo RRT, starting one hour from the start of the minocycline infusion through 48 hours post infusion, per the Manual of Procedures.

Medications history (concomitant medications) will include a review of all medications taken within 24 hours prior to start of infusion, through 48 hours post infusion, as well as the SAE follow-up visit as needed. Pertinent data for concomitant medications will be documented per the Manual of Procedures. Per the exclusion criteria, the following medications will also be documented:

Isotretinoin within 2 weeks prior to enrollment

Tetracycline class drugs within 7 days prior to enrollment

Any investigational product within 7 days prior to enrollment

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. All physical examinations will be performed by a study clinician licensed to make medical diagnoses.

Vital signs, including systolic and diastolic blood pressures (mm Hg), heart rate (HR), respiratory rate, and temperature will be recorded at the indicated time points. Please refer to the Manual of Procedures for details.

Height and weight will be measured or calculated per the Manual of Procedures, rather than estimated or by report of relative.

8.2 Laboratory Evaluations

8.2.1 Clinical Laboratory Evaluations

Hematology, clinical chemistry, and urinalysis will be carried out according to standard operating procedures used by the clinical laboratory at each site.

Each site will maintain a list of the normal ranges and units of measurement for the laboratory parameters to be determined during this study, and the data and certification number of the laboratory. If the normal ranges change during the course of the study, the site investigator must update this list with the new ranges and effective dates. The normal laboratory reference ranges will also be retained in the Regulatory Binder at each site. Laboratory tests will be obtained from screening through the final study visit (48 hours). Refer to the Schedule of Events located in Appendix A. The laboratory tests to be performed are listed below.

Hematology - hemoglobin, hematocrit, white blood cell count (WBC), WBC differential, red blood cell count, platelets

Clinical chemistry - potassium, sodium, chloride, bicarbonate, glucose, blood urea nitrogen, serum creatinine

Liver function tests: AST, ALT, alkaline phosphatase, albumin, total bilirubin

Magnesium

Serum hCG pregnancy test (females of child bearing potential only)

Urinalysis - protein, glucose, ketones, bilirubin, blood, nitrites, LCE, urobilinogen, specific gravity, and pH

8.2.2 Special Assays or Procedures

Pharmacokinetic blood samples will be collected at the appropriate time points. Plasma will be separated and stored frozen, preferably at -80°C; however, use of a -20°C freezer prior to shipping to central lab is permissible but must not exceed 2 weeks. Plasma PK samples will be analyzed for both free and total minocycline concentrations in plasma using a validated liquid chromatography assay with mass spectrophotometry detection (LC-MS). Please refer to the Manual of Procedures for more complete details.

8.2.3 Specimen Preparation, Handling, and Shipping

8.2.3.1 Instructions for Specimen Preparation, Handling, and Storage

Specimen preparation, handling and storage instructions are included in the Manual of Procedures.

8.2.3.2 Specimen Shipment

Specimen shipment instructions are included in the Manual of Procedures.

9 ASSESSMENT OF SAFETY

9.1 Specification of Safety Parameters

Minocin IV is an approved drug with an established safety profile. As such, no safety outcomes are assessed through this study.

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

9.2.1 Adverse Events

In this study a single 200 mg infusion of Minocin IV is being administered. As this is an approved product at an approved dose, and the study is being performed in subjects in the ICU who will have multiple events due to their underlying disease, only SAEs will be captured in this protocol.

9.2.2 Serious Adverse Events

An adverse event is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes:

Death,

A life-threatening adverse event,[2]

Inpatient hospitalization or prolongation of existing hospitalization,

A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or

A congenital anomaly/birth defect.

Important medical events that may not result in death, be life-threatening, or require hospitalizations may be considered serious when, based upon appropriate medical judgment they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias

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

or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

All SAEs will be:

recorded on the appropriate SAE eCRF

followed through resolution by a study clinician

reviewed and evaluated by a study clinician

SAEs will be collected from the start of the investigational drug infusion and will continue for 24 hours from that point. As the study population in this protocol are critically ill patients, the utility of SAE collection (i.e., the ratio between true safety signal and all events that meet the SAE criteria), might be low. The study team will evaluate the utility of SAE collection on a regular basis and will consider change in duration, scope or intensity of SAE collection. Any changes in SAE collection will only be implemented following a protocol amendment.

Timing of Recording and Reporting of SAEs

Serious Adverse Events (SAEs) will be recorded and reported from the start of the Minocin IV infusion until 24 hours from the start of infusion. All SAEs will be followed until resolution or stabilization. Resolution of an SAE is defined as the return to baseline or stabilization of the condition with the expectation that it will remain chronic.

All SAEs must be graded for severity and relationship to study product.

Severity of Event:

SAEs will be assessed by a licensed study physician listed on the Form FDA1572 as the site principal investigator or appropriate sub-investigator. 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.

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

Relationship to Study Products:

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 the study. If there is any doubt as to whether a clinical observation is an SAE, the event should be reported. All SAEs must have their relationship to study product assessed 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.

9.2.3 Expectedness

Expectedness will be based on adverse events based on the current label.

9.3 Reporting Procedures

All SAEs that occur from the start of infusion through 24 hours after the start of infusion will be reported.

9.3.1 Serious Adverse Events

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

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

DMID Pharmacovigilance Group
Clinical Research Operations and Management Support (CROMS)
6500 Rock Spring Dr. Suite 650
Bethesda, MD 20814, USA
SAE Hot Line: 1-800-537-9979 (US) or 1-301-897-1709 (outside US)
SAE FAX Phone Number: 1-800-275-7619 (US) or 1-301-897-1710 (outside US)
SAE Email Address: PVG@dmidcroms.com

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

The DMID medical monitor and clinical protocol manager will be notified of the SAE by the DMID Pharmacovigilance Group. The DMID medical monitor will review and assess the SAE for regulatory reporting and potential impact on study subject safety and protocol conduct.

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

9.3.2 Regulatory Reporting for Studies Conducted Under DMID-Sponsored IND

Following notification from the investigator, DMID, the IND sponsor, will report any suspected adverse reaction that is both serious and unexpected. DMID will report an adverse event as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the drug and the adverse event. DMID will notify FDA and all participating investigators (i.e., all investigators to whom the sponsor is providing drug under its INDs or under any investigator's IND) in an IND safety report of potential serious risks from clinical trials or any other source, as soon as possible, but in no case later than 15 calendar days after the sponsor determines that the information qualifies for reporting as specified in 21 CFR Part 312.32. DMID will also notify FDA of any unexpected fatal or life-threatening suspected adverse reaction as soon as possible but in no case later than 7 calendar days after the sponsor's initial receipt of the information. Relevant follow up information to an IND safety report will be submitted as soon as the information is available. Upon request from FDA, DMID will submit to FDA any additional data or information that the agency deems necessary, as soon as possible, but in no case later than 15 calendar days after receiving the request.

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

9.3.3 Reporting of Pregnancy

Pregnancy occurring during a clinical investigation, although not considered a SAE, must be reported within the same timelines as an SAE. The positive pregnancy test should be recorded in the database within 5 days of site awareness, on the Pregnancy Report form. The report will include pregnancy outcome (e.g., any premature terminations, elective or therapeutic, any spontaneous abortions or stillbirths), as well as the health status of the mother and child, including date of delivery and infant's sex and weight. Pregnancies will be followed until birth. If the database is locked at time of pregnancy, a supplemental report will be generated and completed after birth, which will be appended to the database. Any occurring AEs or SAEs that occur to the mother or fetus will be recorded in the AE or SAE eCRF in the database.

Sites are responsible for notifying their local IRBs of any pregnancies in accordance with local policies.

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

All SAEs will be followed until resolution or stabilization.

9.5 Halting Rules

Further enrollment will be halted for safety monitoring committee (SMC) review/recommendation if any SAE meets the SUSAR criteria.

9.6 Safety Oversight (SMC)

Safety oversight will be conducted by a SMC which is an independent group of experts that monitors subject safety and advises DMID. Members of the SMC will be separate and independent of study personnel participating in this study and should not have scientific, financial or other conflict of interest related to the study. The SMC will consist of at least 3 voting members with appropriate expertise to contribute to the interpretation of the safety data from this trial. In addition, DMID can request the ad-hoc review of safety events by a local independent assessor at the clinical site

The SMC meetings for data review are as follows:

Organizational meeting (prior to start of the study)

Data Review Meetings (DRM) will be held during the study:

An ad hoc SMC meeting will be convened when a halting rule is met, or at the request of the investigator and/or DMID if there are safety concerns during the course of the study.

Annually for aggregate review of SAEs.

Final Data Review Meeting: 6-8 months after clinical database lock to review the cumulative unblinded safety data for this trial. The data will be provided in a standard summary format.

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

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

10 CLINICAL MONITORING

A separate clinical monitoring plan will be developed to describe who will conduct the monitoring, what frequency of monitoring will be done, and what level of detail monitoring will be performed.

10.1 Site Monitoring Plan

Site monitoring is conducted to ensure that the human subject protection, study procedures, laboratory, study intervention administration, and data collection processes are of high quality and meet the sponsor, ICH E6 and, when appropriate, regulatory guidelines. This section provides a general description of how site monitoring will be conducted. A separate monitoring plan document will be developed to describe who will conduct the monitoring, at what frequency monitoring will be done, and what level of detail monitoring will be conducted.

The monitoring plan must include the number of subject charts to be reviewed, which/what proportion of data fields and what will be monitored, and who will be responsible for conducting the monitoring visits.

The sponsor has ethical, legal and scientific obligations to carefully follow this study in accordance with established research principles and applicable regulations. The investigator, as part of his responsibilities, is expected to cooperate with the Sponsor in ensuring that the study adheres to the protocol and GCP requirements.

As part of a concerted effort to fulfill these obligations, the sponsor's monitor (or designee) will visit the center(s) during the study in accordance with the Monitoring Plan set forth for this trial. The investigator will permit the Sponsor to monitor the study as frequently as is deemed necessary and provide access to medical records/source documents to ensure that data are being recorded adequately, that data are verifiable and that protocol adherence is satisfactory.

11 STATISTICAL CONSIDERATIONS

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

This is an observational, single-arm, single-dose, open-label study of the pharmacokinetics and pharmacodynamics of Minocin IV in up to 50 ICU patients who are already receiving antimicrobial therapy for a known or suspected Gram-negative infection. Each subject will receive a single 200mg dose of Minocin IV infused in normal saline over approximately 60 minutes, after which the subject will have PK samples collected at designated time points over a ~48 hour period following the Minocin IV infusion. 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 Manual of Procedures.

For PK specimens collection and processing details, please refer to the Manual of Procedures.

11.1 Study Hypotheses

The aim of the study is to characterize the PK of minocycline, administered as a single dose of Minocin IV, in critically ill subjects. As such, no formal hypothesis testing will be performed. The final population PK model will generate a population mean for each PK parameter; statistical inference will consist of associated 95% confidence intervals (CIs) around the parameter estimates, including estimates of the effects of patient-level and clinical covariates.

The final population PK model will also be used to generate individual patient-specific PK parameters (CL, Vc, CLd and Vp) which will be used to calculate C_{max}, $AUC_{0\text{-}24}$, $AUC_{0\text{-}last}$, and $AUC_{0\text{-}inf.}$and C_{24}.

To assess the probability of attaining PK-PD targets associated with microbiologic efficacy (achievement of fAUC:MIC targets associated with bacterial stasis and 1-log kill for *A. baumannii*) , Monte Carlo simulations will be conducted. Simulations may also be used to assess the probability of target attainment for specific subgroups of patients and for alternate dosing strategies.

11.2 Sample Size Considerations

The sample size for this study was selected in accordance with current guidance for PK studies.[11]

11.3 Planned Interim Analyses

No interim analyses are planned. A safety summary report will be generated for SMC review if the halting rule (see Section 9.5) is met.

11.3.1 Safety Review

If the halting rule (see Section 9.5) is met, a safety summary report (SSR) will be produced and sent to the SMC. The content and format of the SSR will be defined in the SMC charter.

11.4 Final Analysis Plan

11.4.1 Physiological Measures

A shift table describing change from baseline at the 48 hour post-dose time point will be provided for liver function tests, magnesium and serum creatinine.

11.4.2 Pharmacokinetic Analysis and Assessment of Pharmacokinetic-Pharmacodynamic Target Attainment

The methodology used to conduct the PK and PK-PD target attainment analyses will be described in detail in a formal SAP and is briefly outlined in the sections below.

Initial Exploratory Analysis. Box and whisker plots will be used to explore the potential for any outliers in the observed concentration-time dataset. Queries will be generated to resolve potential erroneous time or concentration data point entries due to transcription or measurement errors. Individual concentration-time plots presented on both a linear and semi-log scale will be visualized to ascertain the general structure that may best model the population profile.

Non-compartmental pharmacokinetic analysis. Non-compartmental analysis (NCA) will serve as the initial approach to generate base PK parameter estimates for both the free and total minocycline concentration-time data. An appropriate statistical analysis software package such as R will be used to execute the NCA. This descriptive analysis will permit direct comparisons of the PK data generated from the present study to those generated through studies conducted several decades ago. The following PK parameters will be calculated: maximum plasma concentration (C_{max}), plasma concentration at 24 hours after a dose (C_{24}), time to C_{max} (T_{max}), area under the curve from time zero to 12 hours ($AUC_{0–12}$), 24 hours ($AUC_{0–24}$), the last quantifiable sample ($AUC_{0–last}$) and infinity ($AUC_{0–\infty}$). Actual infusion and sampling times will be used in the calculation of the PK parameters.

Population Pharmacokinetic Analysis. Population pharmacokinetic analysis provides a platform to identify patient covariates which can help explain a portion of the interindividual variability in selected PK parameters. The non-linear mixed effects modeling software NONMEM Version 7.3 (ICON Development Solutions, Ellicott City, MD) will be used to develop the population PK model for both free and total minocycline concentrations in plasma. The first-order conditional estimation method with interaction (FOCEI) will be utilized; other estimation methods such as expectation-maximization (e.g., SAEM) will also be considered.

The initial approach for development of a base structural model will be to co-model the free and total plasma minocycline concentration-time data, assuming both can be adequately characterized using a linear two-compartment model with zero-order infusion as has been used previously.[12] Other model modifications will be considered as necessary. Upon selection of an appropriate base structural PK model, patient covariate effects will be evaluated using stepwise forward selection (α=0.01) followed by stepwise backward elimination (α=0.001) processes.

Monte Carlo Simulation

Using the final population PK model including all statistically significant covariate effects, Monte Carlo simulation will be performed to generate plasma minocycline concentration time data in a virtual population of critically ill patients with gram negative infections. Minocycline doses of 200 mg given as a 1-hour IV infusion, will be evaluated. The total drug and free-drug area under the plasma minocycline concentration-time curve values will then be calculated for each simulated patient using numerical integration. As a quality-control check, the simulated median and 90% prediction interval will be overlaid upon the observed plasma minocycline concentration-time data from critically-ill patients in this study where applicable to ensure the simulated data agree with the observed data.

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 free-drug AUC:MIC ratio (*f*AUC:MIC). 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 across four different clinical isolates of *A. baumannii* studied in a rat lung pneumonia infection model.[13]

The percent of simulated patients achieving the *f*AUC:MIC target of either 12 or 18 will minimally be evaluated for the FDA approved IV minocycline dosing regimen of 200 mg simulated and assessed over a range of MIC values for *A. baumannii.* Recent surveillance MIC data will be obtained to assess whether the PK-PD target attainment is adequate for the majority of the observed MIC distribution for *A. baumannii.*

12 SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA/DOCUMENTS

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

13 QUALITY CONTROL AND QUALITY ASSURANCE

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

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

14 ETHICS/PROTECTION OF HUMAN SUBJECTS

14.1 Ethical Standard

The investigator will ensure that this study is conducted in full conformity with the principles set forth in The Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research of the US National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research (April 18, 1979) and codified in 45 CFR Part 46 and/or the ICH E6; 62 Federal Regulations 25691 (1997).

14.2 Institutional Review Board

Prior to enrollment of subjects into this trial, the approved protocol and informed consent form will be reviewed and approved by the appropriate IRB listed on its Federal Wide Assurance (FWA). The responsible official for the IRB will sign the IRB letter of approval of the protocol prior to the start of this trial and a copy will be provided to DMID. The IRB FWA number will be provided to DMID.

Should amendments to the protocol be required, the amendments will be approved by the sponsor and provided to the site principal investigator for submission to the IRB.

14.3 Informed Consent Process

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continuing throughout the individual's study participation. Extensive discussion of risks and possible benefits of this therapy will be provided to the subject/LAR. Consent forms describing in detail the study interventions/products, study procedures, and risks are given to the subject/LAR and written documentation of informed consent is required prior to starting intervention/administering study product. Consent forms will be IRB-approved and the subject/LAR will be asked to read and review the document. Upon reviewing the document, the investigator will explain the research study to the subject/LAR and answer any questions that may arise. The subject/LAR will sign the informed consent document prior to any procedures being done specifically for the study. The subject/LAR should have the opportunity to discuss the study with their surrogates or think about it prior to agreeing to participate. The subject/LAR may withdraw consent at any time throughout the course of the trial. A copy of the informed consent document will be given to the subject/LAR for their records. The rights and welfare of the subjects will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

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

This study will enroll individuals who are receiving care in an ICU. The target population will be adults including all minorities. This is the first time this product is being tested in this critically ill population, and as such, pregnant women and children will not be able to be included.

14.5 Subject Confidentiality

Subjects will have code numbers and will not be identified by name. Subject confidentiality is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their agents. This confidentiality is extended to cover testing of biological samples and the clinical information relating to participating subjects.

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

All information provided by the Sponsor and all data and information generated by the participating sites as part of this trial (other than a subject's medical records) will be kept confidential by the site principal investigator and other study personnel to the extent permitted by law. This information and data will not be used by the site principal investigator or other study personnel for any purpose other than conducting this trial. These restrictions do not apply to: (1) information which becomes publicly available through no fault of the site principal investigator or other study personnel; (2) information which is necessary to disclose in confidence to an IRB solely for the evaluation of this trial (3) information which is necessary to disclose in order to provide appropriate medical care to a study subject; or (4) study results which may be published as described in Section 16. If a written contract for the conduct of this trial which includes confidentiality provisions inconsistent with this statement is executed, that contract's confidentiality provisions shall apply rather than this statement.

The study monitor or other authorized representatives of the sponsor may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the subjects in this study. The clinical study site will permit access to such records.

14.6 Study Discontinuation

If the trial is discontinued, subjects who sign the informed consent form, and who receive study drug will continue to be followed for safety, as per the schedule of events.

14.7 Future Use of Stored Specimens

No samples will be retained for future testing purposes.

15 DATA HANDLING AND RECORD KEEPING

The investigator is responsible to ensure the accuracy, completeness, legibility, and timeliness of the data reported

Data collection forms will be derived from the eCRFs and provided by the SDCC to the sites to record and maintain data for each subject enrolled in the study. All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data. Permanent ink is required to ensure clarity of reproduced copies. When making a change or correction, the original entry should be crossed out with a single line, and the change should be initialed and dated. Do not erase, overwrite, or use correction fluid or tape on the original.

Data reported in the eCRF should be consistent with the data collection form/source documents or the discrepancies should be documented.

15.1 Data Management Responsibilities

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

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

The Emmes Corporation will serve as the Statistical and Data Coordinating Center for this study and will be responsible for data management, quality review, analysis, and reporting of the study data.

15.2 Data Capture Methods

Clinical data (including SAEs and concomitant medications data) and clinical laboratory data will be entered into a 21 CFR Part 11-compliant Internet Data Entry System (IDES) provided by The Emmes Corporation. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the data collection forms completed by the study personnel.

15.3 Types of Data

Data for this study will include safety and laboratory (pharmacokinetic) data.

15.4 Timing/Reports

A final report will be prepared following the availability of all the clinical, safety, and pharmacokinetic data. Safety data will be provided to the SMC and investigators as per the SMC charter.

15.5 Study Records Retention

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

15.6 Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol, Good Clinical Practice (GCP), or Manual of Procedures requirements. The noncompliance may be either on the part of the subject, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

These practices are consistent with ICH E6:

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

5.1 Quality Assurance and Quality Control, section 5.1.1

5.20 Noncompliance, sections 5.20.1, and 5.20.2.

It is the responsibility of the site to use continuous vigilance to identify and report deviations within 5 working days of identification of the protocol deviation, or within 5 working days of the scheduled protocol-required activity. All deviations must be promptly reported to DMID, via The Emmes Corporation's IDES.

All deviations from the protocol must be addressed in study subject source documents. A completed copy of the DMID Protocol Deviation Form (IDES form) must be maintained in the regulatory file, as well as in the subject's source document. Protocol deviations must be sent to the local IRB per their guidelines. The site PI/study staff is responsible for knowing and adhering to their IRB requirements.

16 PUBLICATION POLICY

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

The International Committee of Medical Journal Editors (ICMJE) member journals have adopted a trials-registration policy as a condition for publication. This policy requires that all clinical trials be registered in a public trials registry such as ClinicalTrials.gov*, which is sponsored by the National Library of Medicine. Other biomedical journals are considering adopting similar policies. It is the responsibility of DMID to register this trial in an acceptable registry. Any clinical trial starting enrollment after 01 July 2005 must be registered on or before subject enrollment. For trials that began enrollment prior to this date, the ICMJE member journals will require registration by 13 September 2005, before considering the results of the trial for publication.

The ICMJE defines a clinical trial as any research project that prospectively assigns human subjects to intervention or comparison groups to study the cause-and-effect relationship between a medical intervention and a health outcome. While studies designed for other purposes, such as to study pharmacokinetics or major toxicity (e.g., Phase I trials), are exempt from this policy, a separate NIH policy requires the registration and reporting in ClinicalTrials.gov for all trials receiving NIH funding for studies starting as of January 18, 2017.

All publications resulting from this study will be reviewed by DMID prior to submittal for publication. All presentations, abstracts, or manuscripts will be reviewed by DMID prior to submission or presentation.

NIH contract support will be acknowledged in all publications.

*Journal Citation:

De Angelis C, Drazen JM, Frizelle FA, Haug C, Hoey J, Horton R, et al. Clinical trial registration: a statement from the International Committee of Medical Journal Editors. N Engl J Med. 2004;351:1250-1.

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SUPPLEMENTS/APPENDICES

APPENDIX A: SCHEDULE OF EVENTS

Table 1: Schedule of Events

	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**	

Footnotes

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