UNIKLINIK KÖLN
Institut für Medizinische Statistik, Informatik und Epidemiologie

Lehr- und Forschungsgebiet
Medizinische Statistik und Epidemiologie
Prof. Dr. Martin Hellmich

Statistical Analysis Plan

Study title:	A Phase 1, Randomized, Double-Blind, Placebo-Controlled, Single and Multiple Ascending Dose Study of the Safety, Tolerability, Pharmacokinetics of Minocin® (minocycline) for Injection in Healthy Adult Subjects
Study code:	Minocin 701 EudraCT No.: 2016-002246-24
Indication:	None (healthy subjects)
Investigational intervention:	Minocin® (minocycline) for Injection
Comparator:	Placebo
Sponsor (or representative):	The Medicines Company 8 Sylvan Way Parsippany, NJ 07054
Financial support:	---
Protocol identification:	MDCO-MIN-16-02
Development phase:	Phase 1
Principal investigator:	Christine Voors-Pette QPS Netherlands BV Petrus Campersingel 123 Groningen, 9713 AG The Netherlands
Statistics:	Stefanie Hamacher
SAP author:	Stefanie Hamacher, Susanne Steinhauser University Hospital of Cologne (UKK) Institute of Medical Statistics, Informatics and Epidemiology (IMSIE) Kerpener Str. 62 50937 Koeln, Germany

CONFIDENTIAL

SAP version: 03 (final) Date: 23.02.2018

version	changes
03	1.2: update of (planned) cohorts
03	6.1: criteria for ITT population

Approved by

Oliver Cornely, MD Medical Lead	01/03/2018	
	Date	Signature
Stefanie Hamacher, MSc Statistician	28.02.2018	S. Hamacher
	Date	Signature
Shu Zhang, PhD Statistician	2/26/18	
	Date	Signature

Content

1 Background

1.1 Trial objective

The primary objective is to assess the safety and tolerability of single and multiple intravenous doses of Minocin IV when administered to healthy adult subjects.
The secondary objective is to assess the pharmacokinetics of single and multiple intravenous doses of Minocin IV when administered to healthy adult subjects.

1.2 Trial design

This is a double-blind, randomized, placebo-controlled, single- and multiple ascending dose study of up to 6 doses/cohorts of Minocin IV.
Each cohort will consist of 10 subjects (8 active drug (at least 2 male and 2 female) and 2 placebo (1 male and 1 female)).
Planned Cohorts:

- Cohort 1: 100 mg*
- Cohort 2: 200 mg*
- Cohort 3: 300 mg
- Cohort 4: 400 mg
- Cohort 5: 500/300 mg
- Cohort 6: 600/300 mg

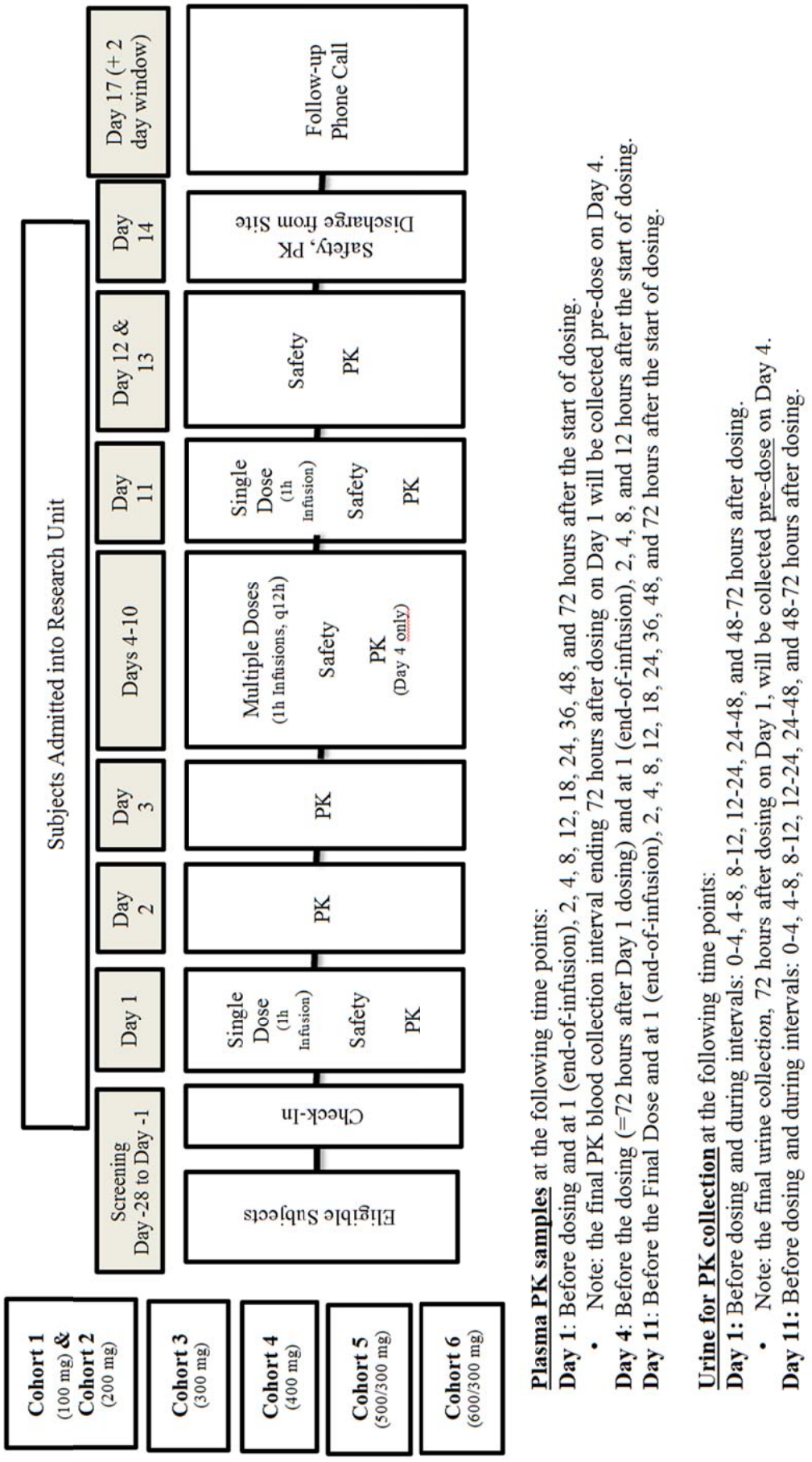
* Cohorts 1 and 2 may be run concurrently as they are the approved doses of minocycline IV in the US. Cohorts 1/2, 3, 4, 5 and 6 will be run sequentially. Additional/replacement cohorts (e.g. due to dosing errors) will be presented as separate cohorts.
Within each cohort, subjects will receive a single dose on Day 1, followed by 7 days of multiple-doses (Days 4-10), followed by a single dose on Day 11. Subjects will be discharged from the clinic on Day 14 upon having an EOS evaluation and follow-up phone call will occur on Day 17.
Subjects are required to remain admitted in the research unit from the time of randomization (Day 1) until discharge on Day 14. Subjects that discontinue the study early are required to complete Early Termination procedures.
Safety will be assessed throughout the study and serial blood and urine samples will be collected for the safety and PK assessment of Minocin IV.
Adverse events and serious adverse events will be collected and recorded. Safety will be evaluated by the assessment of clinical safety laboratory results, vital sign measurements, ECG and physical examination findings. A Day 17 follow up phone call will be performed to evaluate safety by the assessment of adverse events and serious adverse events.
The estimated study period will be approximately 25 weeks from screening of the first subject to completion of the last subject.

The schedule of events/assessments is given in Figure 1-1 and a schematic diagram of trial design is shown in Figure 1-2.

Study Procedures	Screening (≤28 days to -1)	Day -1[1]	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14 (EOS /ET)	Day 17 (+2 days)
Informed consent	X																
Medical History & Demographics	X	X															
Height, weight and BMI[3]	X	X										X				X	
Physical Examination	X	X			X						X					X	
Vital Signs:RR/BP/HR/Temperature[4]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
ECG (12-lead)[5]	X	X	X			X	X	X	X	X	X	X	X			X	
Laboratory Assessments[6]	X	X		X		X		X		X		X			X	X	
Creatinine clearance[7]			X														
Test for HIV, Hep B sAg, HCV Ab	X																
Urine Drug Screen / Alcohol breath test[8]	X	X															
Pregnancy Test[9]	X	X	X													X	
PK Blood Collection[10]			X	X	X	X[2]							X	X	X	X	
PK Urine Collection[11]			X	X	X	X							X	X	X	X	
Inclusion / Exclusion Criteria	X	X															
Dose Administration (1h Infusion)[12]			X			X	X	X	X	X	X	X	X				
Concomitant Medications	<---X--->																
Adverse Events / SAEs	<---X--->																
Follow-Up Phone Call																	X

[1] Day -1 procedures should be completed within approximately 24 hours before dosing on Day 1.
[2] The 72 h post-dose PK collection should occur before the start of the 2nd infusion on Day 4.
[3] BMI will only be calculated at screening. Weight will be collected on Day -1, 10 and 14.
[4] Vital signs (blood pressure, pulse, temperature, respirations) will be measured before and at 1, 3, and 6 h after the start of the morning dose and before and at 1 hour after the start of the evening dose. On Day 1 vitals will be collected at 1, 3, and 6 h after the start of dosing. Vital signs may be collected once in the morning on days where no dosing occurs.
[5] On Day -1, ECGs should be performed in triplicate. On Days 1 a triplicate ECGs will be performed 1h end of infusion (+/- 10 minutes). Day4 triplicate ECGs will be performed before (- 1 hour) and 1 h end of infusion (+/-10 minutes) after the start of the morning dosing. On Days 5-11 and 14, single ECGs are performed. Triplicate recording may be required depending on Baseline findings; see 11.1.7.
[6] Includes serum chemistry, hematology, and urinalysis. On Days 4, 6, 8, and 10, collect blood and urine for clinical laboratory tests approx. 4 h after the start of the morning dose.
[7] Urine from the PK collection on Day 1 (hours: 0-4, 4-8, 8-12, 12-24) will also be used to determine 24h creatinine clearance.
[8] Required for all subjects.
[9] Serum pregnancy should be performed during Screening, Day -1 and Day 14 EOS. Urine pregnancy test should be performed before dosing on Day 1.
[10] Serial plasma PK samples at the following time points (in hours) on **Day 1**:Before dosing and at 1 (end-of-infusion), 2, 4, 8, 12, 18, 24, 36, 48, and 72 h (pre-dose on Day 4) after the start of dosing; Post-**Day 4 dose**: 1 (end-of-infusion), 2, 4, 8, and 12 h after the start of dosing; On **Day 11**: Before the Final Dose and at 1 (end-of-infusion), 2, 4, 8, 12, 18, 24, 36, 48, and 72 h after the start of dosing.
[11] Urine for PK analysis will be collected at **Day 1** before dosing and during intervals: 0-4, 4-8, 8-12, 12-24, 24-48, and 48-72 h (pre-dose on Day 4) after dosing. **Day 11** before dosing and during intervals: 0-4, 4-8, 8-12, 12-24, 24-48 and 48-72 hours after dosing.
[12] Subjects will receive a single dose on Day 1. Dosing on Days 4-10 will occur every 12 h. Only the one morning dose on Day 11 will be administered.

Figure 1-1: Schedule of events/assessments



Plasma PK samples at the following time points:

Day 1: Before dosing and at 1 (end-of-infusion), 2, 4, 8, 12, 18, 24, 36, 48, and 72 hours after the start of dosing.

- Note: the final PK blood collection interval ending 72 hours after dosing on Day 1 will be collected pre-dose on Day 4.

Day 4: Before the dosing (=72 hours after Day 1 dosing) and at 1 (end-of-infusion), 2, 4, 8, and 12 hours after the start of dosing.

Day 11: Before the Final Dose and at 1 (end-of-infusion), 2, 4, 8, 12, 18, 24, 36, 48, and 72 hours after the start of dosing.

Urine for PK collection at the following time points:

Day 1: Before dosing and during intervals: 0-4, 4-8, 8-12, 12-24, 24-48, and 48-72 hours after dosing.

- Note: the final urine collection, 72 hours after dosing on Day 1, will be collected pre-dose on Day 4.

Day 11: Before dosing and during intervals: 0-4, 4-8, 8-12, 12-24, 24-48, and 48-72 hours after dosing.

Figure 1-2: Schematic diagram of trial design

1.3 Assessment of Safety

Intra-Cohort Data Review

For Cohorts 3-6, single dose safety data will be reviewed by the Medical Monitor and Principal Investigator on Day 3 prior to the start of the multi- dosing on Day 4. During the review, blinded safety data from the single dose infusions will be assessed and one of the following will be decided before the start of the Multiple Dose Phase on Day 4:

- To continue with the Multi-dose phase as planned,
- To continue with the Multi-dose phase and add additional safety evaluations,
- To not continue with the Multi-dose phase.

Between-Cohort Data Monitoring Committee Review

Safety data and PK data will be reviewed by the DMC following each cohort and prior to the start of the next cohort.

After completion of cohorts 1 and 2, which may run concurrently, and prior to dosing of each cohort 3, 4, 5 and 6, the DMC will review all pertinent blinded safety data (e.g., physical examinations, vital signs, electrocardiograms (ECGs), clinical laboratory tests, and AEs) from the preceding cohort to determine one of the following:

- To continue with the next cohort as planned
- To continue with the next cohort and add additional safety evaluations
- To continue with the next cohort by adjusting the planned dose of Minocin IV
- To not continue with the next cohort

Stopping Rules

The study will be terminated if ≥ 2 subjects in a cohort meet any of the following criteria and the subject was determined to have received active drug after unblinding:

- Has a drug related SAE.
- Experiences a drug related AE grade > 3 toxicity.

A written statement fully documenting the reasons for study termination will be provided to the HREC/IEC.

1.4 Timing of Analyses

After completion of the last visit of the last subject data will be cleaned and approved by CTCC according to their SOPs. The cleaned data will then be transferred to the statistician and the final analysis will be performed after the finalization and approval of this statistical analysis plan (SAP).

Number of patients in the final dataset is not known and depends on DMC decision on involved cohorts.

2 Analysis populations

2.1 Definitions

The following populations will be used for data analyses and/or presentation.

Intent-to-Treat (ITT) Population

All subjects randomized into the trial. Treatment classification will be based on the randomized treatment.

Modified Intent-to-Treat (mITT) Population

All enrolled subjects who receive at least one dose of study drug. Treatment classification will be based on the randomized treatment.

Pharmacokinetics (PK) Population

All mITT subjects who receive the active study drug and have any valid samples measured for study drug levels. Treatment classification will be based on the actual treatment received. This population will be used for PK analysis.

Safety Population

The safety set will be the mITT population.

2.2 Application

Decision on the analysis populations is made prior data lock and before the study is unblinded.

The safety population will be used for the evaluation of safety and tolerability of Minocin IV. The pharmacokinetic analysis will be conducted in the PK population.

2.3 Major protocol violations / Withdrawals

Withdrawals are included at least in the ITT population. If they receive any study medication they are included in mITT and safety as well. The drop-outs will be listed with group, time and reason.

3 Trial centers

This clinical trial will be carried out at a single study site:

QPS Netherlands BV
Petrus Campersingel 123
Groningen, 9713 AG
The Netherlands

The planned sample size is 10 subjects per cohort for all 5 cohorts. Each randomized subject can be part of one cohort only, i.e. cannot be randomized twice.

4 Analysis variables

4.1 Demography and baseline characteristics

The demographic and baseline variables will be measured during screening period (day -28 to -1), during check-in procedure (day -1) or pre-dose on Day 1.
The following demographic variables will be analyzed:

- Age [years]
- Sex [M, F]
- Race [White (White), Black (Black or African American), Asian (Asian), Hawai (Native Hawaiian or Other Pacific Islander), Indian (American Indian or Alaska Native), Other (Other)]

The following baseline variables will be analyzed:

- Physical Examination
    - Height [cm]
    - Weight [kg]
    - BMI [kg/m^2]
    - Skin [normal, abnormal, not done; clinical significant]
    - Head [normal, abnormal, not done; clinical significant]
    - Ears, eyes, nose and throat [normal, abnormal, not done; clinical significant]
    - Respiratory system [normal, abnormal, not done; clinical significant]
    - Cardiovascular system [normal, abnormal, not done; clinical significant]
    - Gastrointestinal system [normal, abnormal, not done; clinical significant]
    - Neurological condition [normal, abnormal, not done; clinical significant]
    - Blood and lymphatic system [normal, abnormal, not done; clinical significant]
    - Musculoskeletal system [normal, abnormal, not done; clinical significant]
    - Other [normal, abnormal, not done; clinical significant]
- Vital Signs
    - Systolic Blood Pressure [mmHg]
    - Diastolic Blood Pressure [mmHg]
    - Pulse rate [beats/min]
    - Respiratory rate [breaths/min]
    - Temperature [°C]

- 12-lead-ECG
    - ECG result [normal, clinically insignificant abnormality, clinically significant abnormality, not assessable]
    - ECG Mean Ventricular Rate [beats/min]
    - PR Interval [msec]
    - QRS Duration [msec]
    - QT Interval, corrected [msec]
    - QT Interval, uncorrected [msec]
- Hematology
    - Hemoglobin [g/dL; clinical significant]
    - Hematocrit [%; clinical significant]
    - White blood cell count [x10^-9/l; clinical significant]
    - Neutrophils abs. [x10^-9/l; clinical significant]
    - Neutrophils % [%; clinical significant]
    - Eosinophils [x10^-9/l; clinical significant]
    - Basophils [x10^-9/l; clinical significant]
    - Monocytes [x10^-9/l; clinical significant]
    - Lymphocytes [x10^-9/l; clinical significant]
    - Red blood cell counts [x10^-12/l; clinical significant]
    - Platelet count [x10^-9/l; clinical significant]
    - Prothrombin Intl. Normalized Ratio (INR) [clinical significant]
    - Activated partial thromboplastin time (aPTT) [sec; clinical significant]
- Chemistry
    - Blood urea nitrogen [mg/dL; clinical significant]
    - Serum Creatinine [mg/dL; clinical significant]
    - Total Bilirubin [mg/dL; clinical significant]
    - Direct Bilirubin [µmol/L; clinical significant]
    - Alkaline phosphatase [U/L; clinical significant]
    - Aspartate transaminase [U/L; clinical significant]
    - Alanine transaminase [U/L; clinical significant]
    - Albumin [g/L; clinical significant]
    - Total protein [g/L; clinical significant]
    - Glucose [mg/dL; clinical significant]
    - Calcium [mmol/L; clinical significant]
    - Chloride [mmol/L; clinical significant]
    - Sodium [mmol/L; clinical significant]
    - Magnesium [mmol/L; clinical significant]

  - Potassium [mmol/L; clinical significant]
  - Uric acid [mg/dL; clinical significant]
  - Lactate dehydrogenase [U/L; clinical significant]
  - Bicarbonate [mmol/L; clinical significant]
  - Phosphorus [mmol/L; clinical significant]
- Urinalysis
  - Protein [negative, positive; clinical significant]
  - Blood [negative, positive; clinical significant]
  - Leucocytes [negative, positive; clinical significant]
  - Nitrite [negative, positive; clinical significant]
  - Glucose [negative, positive; clinical significant]
  - Ketones [negative, positive; clinical significant]
  - Bilirubin [negative, positive; clinical significant]
  - pH [clinical significant]
  - Specific gravity [g/L; clinical significant]
  - Urobilinogen [clinical significant]
  - Additional microscopic exam [yes, no]
- Alcohol and drug screening
  - Alcohol test not done [yes, no]
  - Alcohol test result [negative, positive]
  - Drug test not done [yes, no]
  - Drug test result [negative, positive]
- Pregnancy test
  - Pregnancy test done [yes, no]
  - Pregnancy test not done reason [post-menopausal, surgically sterilized, other]
  - Pregnancy test result [negative, positive]

4.2 Variables for safety and tolerability analysis

The following variables will be used to analyze safety and tolerability of Minocin IV versus placebo:

- Adverse Events (Cumulative)
  - Start date [DD/MM/YYYY]
  - Start time [HH:MM]
  - End date [DD/MM/YYYY]
  - End time [HH:MM]
  - Ongoing at the End of Study [yes, no]
  - Severity of AE [Grade 1, Grade 2, Grade 3, Grade 4, Grade 5]

- Causality to Study Drug [reasonable possibility, no reasonable possibility, unclassified, unclassifiable]
- Action taken with Study Drug [dose not changed, dose reduced, drug withdrawn, na (not applicable)]
- Therapy of Event [yes/no]
- Outcome of Event [recovered/resolved, recovered/resolved with sequelae, recovering/resolving, fatal, unknown]
- Serious Adverse Event [yes/no]
- Date AE turned serious [DD/MM/YYYY]
- Time AE turned serious [HH:MM]
- Results in Death [yes/no]
- Life-threatening [yes/no]
- Results in significant, persistent, … [yes/no]
- Requires in-patient hospitalization or … [yes/no]
- Congenital Anomaly or Birth Defect [yes/no]
- Another medically significant event … [yes/no]

- Laboratory Tests (Day 2, 4, 6, 8, 10, 13, 14)
  - Safety Lab: Hematology
    - Hemoglobin [g/dL; clinical significant]
    - Hematocrit [%; clinical significant]
    - White blood cell count [x10^-9/l; clinical significant]
    - Neutrophils abs. [x10^-9/l; clinical significant]
    - Neutrophils % [%; clinical significant]
    - Eosinophils [x10^-9/l; clinical significant]
    - Basophils [x10^-9/l; clinical significant]
    - Monocytes [x10^-9/l; clinical significant]
    - Lymphocytes [x10^-9/l; clinical significant]
    - Red blood cell counts [x10^-12/l; clinical significant]
    - Platelet count [x10^-9/l; clinical significant]
    - Prothrombin Intl. Normalized Ratio (INR) [clinical significant]
    - Activated partial thromboplastin time (aPTT) [sec; clinical significant]
  - Safety Lab: Chemistry
    - Blood urea nitrogen [mg/dL; clinical significant]
    - Serum Creatinine [mg/dL; clinical significant]
    - Total Bilirubin [mg/dL; clinical significant]
    - Direct Bilirubin [µmol/L; clinical significant]
    - Alkaline phosphatase [U/L; clinical significant]

        - Aspartate transaminase [U/L; clinical significant]
        - Alanine transaminase [U/L; clinical significant]
        - Albumin [g/L; clinical significant]
        - Total protein [g/L; clinical significant]
        - Glucose [mg/dL; clinical significant]
        - Calcium [mmol/L; clinical significant]
        - Chloride [mmol/L; clinical significant]
        - Sodium [mmol/L; clinical significant]
        - Magnesium [mmol/L; clinical significant]
        - Potassium [mmol/L; clinical significant]
        - Uric acid [mg/dL; clinical significant]
        - Lactate dehydrogenase [U/L; clinical significant]
        - Bicarbonate [mmol/L; clinical significant]
        - Phosphorus [mmol/L; clinical significant]
    - Urinalysis
        - Protein [negative, positive; clinical significant]
        - Blood [negative, positive; clinical significant]
        - Leucocytes [negative, positive; clinical significant]
        - Nitrite [negative, positive; clinical significant]
        - Glucose [negative, positive; clinical significant]
        - Ketones [negative, positive; clinical significant]
        - Bilirubin [negative, positive; clinical significant]
        - pH [clinical significant]
        - Specific gravity [g/L; clinical significant]
        - Urobilinogen [clinical significant]
        - Additional microscopic exam [yes, no]
- Vital Signs (Day 1 - Day 14, each: pre-dose, 1h, 3h and 6h post-dose; except Day 1: 1h, 3h and 6h post-dose and Day 2, 3, 12, 13, 14: only once in the morning)
    - Systolic Blood Pressure [mmHg]
    - Diastolic Blood Pressure [mmHg]
    - Pulse rate [beats/min]
    - Respiratory rate [breaths/min]
    - Temperature [°C]
- ECGs (Day 1, 4, 5, 6, 7, 8, 9, 10, 11, 14, each: pre-dose and 1h post-first-dose; except Day 1: 1h post-dose and Day 14 no fixed time point)
    - ECG result [normal, clinically insignificant abnormality, clinically significant abnormality, not assessable]

- ECG Mean Ventricular Rate [beats/min]
- PR Interval [msec]
- QRS Duration [msec]
- QT Interval, corrected [msec]
- QT Interval, uncorrected [msec]

- Physical Examination (Day 3, 9, 14)
  - Height [cm] (only at screening)
  - Weight [kg] (only Day 9, 14)
  - BMI (only Day 9, 14)
  - Skin [normal, abnormal, not done; clinical significant]
  - Head [normal, abnormal, not done; clinical significant]
  - Ears, eyes, nose and throat [normal, abnormal, not done; clinical significant]
  - Respiratory system [normal, abnormal, not done; clinical significant]
  - Cardiovascular system [normal, abnormal, not done; clinical significant]
  - Gastrointestinal system [normal, abnormal, not done; clinical significant]
  - Neurological condition [normal, abnormal, not done; clinical significant]
  - Blood and lymphatic system [normal, abnormal, not done; clinical significant]
  - Musculoskeletal system [normal, abnormal, not done; clinical significant]
  - Other [normal, abnormal, not done; clinical significant]

4.3 PK Variables

Pharmacokinetic data will be analyzed by non-compartmental methods with WinNonlin (version 6.3, Pharsight Corporation, Mountain View, California) and modelled for constant intravenous infusion. The pharmacokinetic parameters calculated will be C_{max} (maximum concentration observed), t_{max} (time at which C_{max} occurs), $AUC_{0\text{-}tlast}$ (area under the curve from time 0 to the last time point), $AUC_{0\text{-}\infty}$ (area under the curve from time 0 extrapolated to infinity), $t_{1/2}$ (half life), CL (plasma clearance), and V_{SS} (volume of distribution). Renal clearance (CL_R) will be calculated as the amount of minocycline excreted into urine divided by the AUC and non-renal clearance will be calculated as the difference between plasma clearance and renal clearance.

For the calculation of summary pharmacokinetic parameters and all analyses of derived pharmacokinetic parameters, C_{max} and V_{ss} values will be normalized for the weight of the subject and expressed as a standardized dose per kg. Continuous variables will be summarized using n, mean, median, standard deviation and range (minimum and maximum). Categorical variables will be summarized using proportions (counts and percentages).

The pharmacokinetic parameters C_{max}, $AUC_{0\text{-}tlast}$ and $AUC_{0\text{-}\infty}$ will be log_e transformed prior to statistical analyses and presented as geometric means in summary output.
No interim analyses, other than for safety purposes, are planned and no imputation or carrying forward of earlier observations will be used for the analyses of data.
For calculations and statistical analysis of pharmacokinetic variables, missing concentrations (either genuinely missing concentrations or below the limit of quantification (BLQ)) measured between two concentrations above the lower limit of quantification (LLOQ)) will be deleted, resulting in an interpolation between the nearest two concentration values.

C_{max}	Maximum concentration in plasma
t_{max}	Time of C_{max} in plasma
$AUC_{0\text{-}t}$	Area under the concentration-time curve from time zero to last quantifiable concentration in plasma
$AUC_{0\text{-}inf}$	AUC extrapolated to infinity in plasma, ($AUC_{0\text{-}t}$ + last quantifiable concentration/ λz)

5 Handling of missing values and outliers

5.1 Missing values

Missing values will not be imputed. Instead, if subjects drop-out before the first dose was given they will be replaced by additionally randomized trial subjects. If subjects drop-out after first dose but before end of study an Early Termination Visit is performed.

5.2 Outliers

For safety, a special outlier handling is not applicable.

6 Statistical analyses / methods

A descriptive statistical analysis will be performed. No tests will be conducted.
Unless stated otherwise, descriptive statistics will include:
Quantitative variables and variables with ordered response categories, will be described by their number of observations (n), arithmetic mean, standard deviation (SD), median, first quartile (Q1), third quartile (Q3), minimum (min) and maximum (max) (see sample Table 12-1). Qualitative variables will be described by their absolute and relative frequencies (see sample Table 12-2).

6.1 Patient / Subject Disposition

First, the application of the inclusion and exclusion criteria to all included subjects will be verified.

Definition of ITT population: All subjects included in the database (eligible for trial, with given written informed consent and randomization ID).

Definition of mITT population: All ITT subjects with number of administered infusions ≥ 1.

Definition of PK population: All subjects with any valid PK blood or urine sampling and number of administered infusions of Minocin > 0.

Definition of safety population: All subjects of the mITT population.

Absolute and relative frequencies of patients per treatment arm and cohort will be calculated for each population. Frequencies will be shown in a subject flow diagram. The diagram will include consent, allocation, follow-up and analysis. Number of and reasons for drop-outs will be included. See sample Figure 12-1 in appendix.

6.2 Demography and baseline characteristics

The last measurement before the first dose (Screening, Day -1 or Day 1) is the baseline value. Demographic and baseline variables will be summarized descriptively by treatment arm, cohort and in total for safety population.

6.3 Prior or concomitant medication and diseases

Medications will be coded using the most updated version of the WHO Drug Dictionary available (e.g. version WHODDE DEC. 1, 2015 or later).

Listings of each prior (pre-baseline) medication and concomitant (baseline or later) medication per treatment group, cohort and patient will be given (see 12.3, 1.). Frequency counts of each prior medication (pre-baseline) and concomitant medication (baseline or later) (according to WHO) will be provided by cohort.

6.4 Study drug administration

Doses not administered per protocol are listed. Infusion administered, Start date, Start time, End date, End time, Reasons for dose administration not per protocol and Residual volume (ml) will be given (see 12.3, 2.).

6.5 Exposition to treatment/Compliance

Number of subjects in the mITT population with number of administered infusions <16 will be calculated per treatment group and cohort.

Listing of drop-outs with reason, treatment group and time (Day 1 - Day 17) will be given (see 12.3, 3.).

6.6 Analysis of safety and tolerability

Variables for the analysis of safety and tolerability will be evaluated in the safety population.

Note: For analysis of adverse events two dosing conditions are defined based on corresponding study day: day 1 to 3 are assigned to single dosing and day 4 to 17 are assigned to multiple dosing. In the following this will be referred to as dosing condition.

6.6.1 Adverse events/Serious adverse events

All AEs occurring from the time when first dose is administered up to the last follow-up visit (phone call on Day 17) are listed by subject with cohort (dose), treatment group, AE number, AE Term, preferred term (MedDRA dictionary), primary system-organ class, study day, dosing condition, start date, start time, end date, end time, ongoing at study end, severity, causality, therapy, therapy specification, outcome and seriousness (see 12.3, 4.).

Additionally, all SAEs will be listed by subject with cohort (dose), treatment group, SAE number, AE number, preferred term (MedDRA dictionary), primary system-organ class, date and time when AE turned serious, SAE diagnosis, results in death, life-threatening, results in significant damage, hospitalization, congenital anomaly or birth defect, another medically significant event and description of event (see 12.3, 5.).

The total number (percentage) of AEs will be tabulated by cohort, treatment group and total, classified by preferred term (MedDRA dictionary), primary system-organ class, severity and causality (see Table 12-3). Even if several AEs are from the same subject, they are counted at subject level.

The absolute and relative frequencies of subjects reporting AEs will be tabulated by cohort and treatment group and classified by:

1. any AE, related AE, unrelated AE, any SAE, related SAE, unrelated SAE (see Table 12-4)
2. each preferred term (MedDRA dictionary) within each primary system-organ class (see Table 12-5)
3. severity within each preferred term (MedDRA dictionary) within each primary system-organ class (see Table 12-6)

If a subject has multiple AEs or SAEs, related and not related to study drug, then the subject is counted as related (‘causality’ = yes). If a subject has multiple AEs/SAEs with the same preferred term, it is counted only once. Likewise, if a subject has multiple AEs with the same preferred term but different severities, the maximal severity is used and the subject is counted only once.

Tables are presented in three versions: all AEs, AEs occurred during single dosing and AEs occurred during multiple dosing.

6.6.2 Laboratory tests (Hematology, Chemistry, Urinalysis)

Laboratory values will be summarized descriptively by treatment group and cohort including mean change and mean percent changes from baseline at each time point where appropriate (see Table 12-7). Shift tables by reference range (see 12.1), which count the number of subjects with a low, normal or high value at baseline and a low, normal or high value at each time point, of each parameter will be given by cohort.

6.6.3 Vital signs

Vital signs will be summarized descriptively by cohort and treatment group including mean change and mean percent change from baseline at each scheduled time point where appropriate (see Table 12-7).

6.6.4 ECGs

Ventricular rate, PR, QRS, QT and QTc intervals will be summarized descriptively by cohort and treatment group for each scheduled time point. If a triplicate measurement was performed the average of the triplicate per subject will be computed and used for analysis.
Furthermore, ECG results are assessed as normal, clinically insignificant abnormal, and clinically significant abnormal. This information will be summarized descriptively by cohort for each scheduled time point. Additionally, a normal-abnormal shift table (see Table 12-8) will be presented for ECGs results for each cohort and treatment group, comparing baseline to each scheduled time point.

6.7 Pharmacokinetic analyses

Pharmacokinetic variables will be evaluated in the PK population.

6.8 Planned subgroup analyses

Not applicable.

6.9 Interim analyses

Not applicable.

7 Deviations from the protocol

Not applicable.

8 Interpretation of results

Because of the small sample size a careful interpretation of the results is essential.

9 Data problems

Not expected. Will be recorded in a separate document.

10 Software

The analyses will be programmed in SAS 9.3 or higher (SAS Institute Inc., Cary, NC, USA). Mainly SAS procedure PROC TABULATE, PROC REPORT and PROC SGPLOT will be used to generate the TFL. Own macros will be validated according to IMSIE SOPs before using them for the analysis.

11 References

Clinical Study Protocol MDCO-MIN-16-02, Version 2.2, Final 07-Apr-17

12 Appendices

12.1 Reference ranges of laboratory parameters

- Activated partial thromboplastin time (aPTT): 24 – 35 sec
- Prothrombin time (PT): 6.7 – 14.8 sec
- Hemoglobin (male): 8.5 – 11 g/dl
- Hemoglobin (female): 7.5 – 10 g/dl
- Hematocrit (male): 0.41 – 0.51 l/l
- Hematocrit (female): 0.36 – 0.47 l/l
- Red blood cell count (male): 4.3 – 5.9 x10^12/l
- Red blood cell count (female): 3.8 – 5.4 x10^12/l
- White blood cell count: 4.0 – 11.0 x10^9/l
- Platelet count: 150 – 400 x10^9/l
- Basophils: <0.2 x10^9/l
- Eosinophils: <0.4 x10^9/l
- Lymphocytes: 0.8 – 3.2 x10^9/l
- Monocytes: 0.3 – 0.9 x10^9/l
- Glucose: 3.5 – 7.8 mmol/l
- Sodium: 135 – 145 mmol/l
- Potassium: 3.5 – 5.0 mmol/l
- Chloride: 97 – 107 mmol/l
- Bicarbonate: 23 – 29 mmol/l
- Serum Creatinine (male): 50 – 110 µmol/l
- Serum Creatinine (female): 50 – 90 µmol/l
- Urea: 2.5 -.7.5 mmol/l
- Lactate dehydrogenase: <250 U/l
- Total protein: 60 – 80 g/l
- Albumin: 35 – 50 g/l
- Total Bilirubin: <17 µmol/l
- Direct Bilirubin: <5 µmol/l
- Alanine transaminase: <45 U/l
- Aspartate transaminase: <40 U/l
- Alkaline phosphatase: <120 U/l
- Uric acid (male): 0.20 – 0.45 mmol/l
- Uric acid (female): 0.15 – 0.35 mmol/l
- Calcium: 2.2 – 2.6 mmol/l

- Magnesium: 0.7 – 1.0 mmol/l
- Phosphorus: 0.8 – 1.4 mmol/l
- Neutrophils: 2 – 7.5 x10^9/l
- pH: 5 – 8
- Specific gravity: 1.002 - 1.035 g/l

12.2 Planned tables

Table 12-1 Table of quantitative demographic.

		Cohort 1	…	Cohort 6	Placebo	Total
Quantitative Variable 1	n					
	mean					
	SD					
	min					
	Q1					
	median					
	Q3					
	max					
Quantitative Variable 2	n					
	mean					
	SD					
	min					
	Q1					
	median					
	Q3					
	max					
…						

Table 12-2 Table of qualitative demographic.

		Cohort 1	…	Cohort 6	Placebo	Total
Total	n					
Qualitative Variable 1						
Expression 1	n					
	c%					
Expression 2	n					
	c%					
Qualitative Variable 2						
Expression 1	n					
	c%					
	…					

Table 12-3 AE tabulation.

		Cohort 1	…	Cohort 6	Placebo	Total
Preferred term	**Term 1**	n (%)		…		
	Term 2	…				
	…					
System-organ class	**SOC 1**					
	SOC 2					
	…					
Severity	**Grade 1**					
	…					
	Grade 5					
Causality	**Reasonable possibility**					
	No reasonable possibility					
Total						

Table 12-4 AE tabulation (version 1).

		Cohort 1	…	Cohort 6	Placebo	Total
AE	**any**	n (%)		…		
	related	…				
	unrelated					
SAE	**any**					
	related					
	unrelated					

Table 12-5 AE tabulation (version 2).

		Cohort 1	…	Cohort 6	Placebo	Total
SOC 1	**Preferred term 1**	n (%)		…		
	Preferred term 2	…				
	…					
SOC 2	**Preferred term 1**					
	Preferred term 2					
	…					
…	**…**					

Table 12-6 AE tabulation (version 3).

System-organ class	Preferred term	Severity	Cohort 1	…	Cohort 6	Placebo	Total
SOC 1	**Term 1**	**Grade 1**	n (%)		…		
		Grade 2					
		Grade 3					
		Grade 4					
		Grade 5					
	Term 2	**Grade 1**	…				
		Grade 2					
		Grade 3					
		Grade 4					
		Grade 5					
	…						
SOC 2	**Term 1**	**Grade 1**					
		Grade 2					
		Grade 3					
		Grade 4					
		Grade 5					
	Term 2	**Grade 1**					
		Grade 2					
		Grade 3					
		Grade 4					
		Grade 5					
	…						
…	**…**						

Table 12-7 Change table for quantitative variables (mean (percent) change from baseline).

		Cohort 1	…	Cohort 6	Placebo	Total
Quantitative Variable 1	n					
	mean					
	SD					
	min					
	Q1					
	median					
	Q3					
	max					
	mean change					
	mean % change					
Quantitative Variable 2	n					
	mean					
	SD					
	min					
	Q1					
	median					
	Q3					
	max					
	mean change					
	mean % change					
…						

Table 12-8 Shift table for qualitative variables.

		Baseline		
		Category 1	**Category 2**	**…**
Day x	**Category 1**	n (%)	…	
	Category 2	…		
	…			
Day y	**Category 1**			
	Category 2			
	…			
…				

12.3 Planned listings

1. Prior or concomitant medication by subject (see 6.3)

Cohort	Treatment Group	ID	Medication (tradename)	WHO drug name	Indication	Total daily dose	Unit
xx	xxx	xxx	xxxxx	xxxxx	xxxxx	xxx	xx

Route of administration	Start date	More than 2 weeks before dosing	End date	Ongoing at study end
xxxxx	dd.mm.yyyy	x	dd.mm.yyyy	x

2. Study Drug Administration (see 6.4)

ID	Cohort	Infusion administered	Start date	Start time	End date	End time
xx	xx	x	dd.mm.yyyy	hh:mm	dd.mm.yyyy	hh:mm

Dose administered per protocol	Reasons for not per protocol	Residual volume (ml)
x	xxxxx	xx

3. Drop-outs with cohort, treatment group, time, reason (Day 1 - 17) (see 6.5)

ID	Cohort	Treatment Group	Time	Reason
xxx	xx	xxx	dd.mm.yyyy	xxxxxxxxxxxxxxx

4. All AEs occurring from administration of first dose up to the last follow-up visit by subject with severity, relationship to study drug, cohort (dose) and treatment group (see 6.6.1)

ID	Cohort	Treatment Group	AE No.	AE Term	Preferred term (MedDRA)	System-organ class	Study day	Dosing condition
xx	xx	xxx	xx	xxxxx	xxxxx	xxxxx	xx	xxxxx

Start date	Start time	End date	End time	Ongoing at the end of study	Severity
dd.mm.yyyy	hh:mm	dd.mm.yyyy	hh:mm	x	xx

Causality	Therapy	Therapy specification	Outcome	Seriousness	CRF comment
xx	x	xxxxx	xx		xxxxx

5. All SAEs occurring from the time when informed consent is obtained at screening up to the last follow-up visit by subject with severity, relationship to study drug, cohort (dose) and treatment group (see 6.6.1)

ID	Cohort	Treatment Group	SAE No.	AE No.	Preferred term (MedDRA)	System-organ class	Date AE turned serious
xx	xxx	xxx	xx	xx	xxxxx	xxxxx	dd.mm.yyyy

Time AE turned serious	SAE diagnosis	Results in death	Life-threatening	Results in significant damage
hh:mm	xxxxx	x	x	x

Hospitalization	Congenital anomaly or birth defect	Another medically significant event	Description of event	CRF comment
x	x	x	xxxxx	xxxxx

12.4 Planned graphics

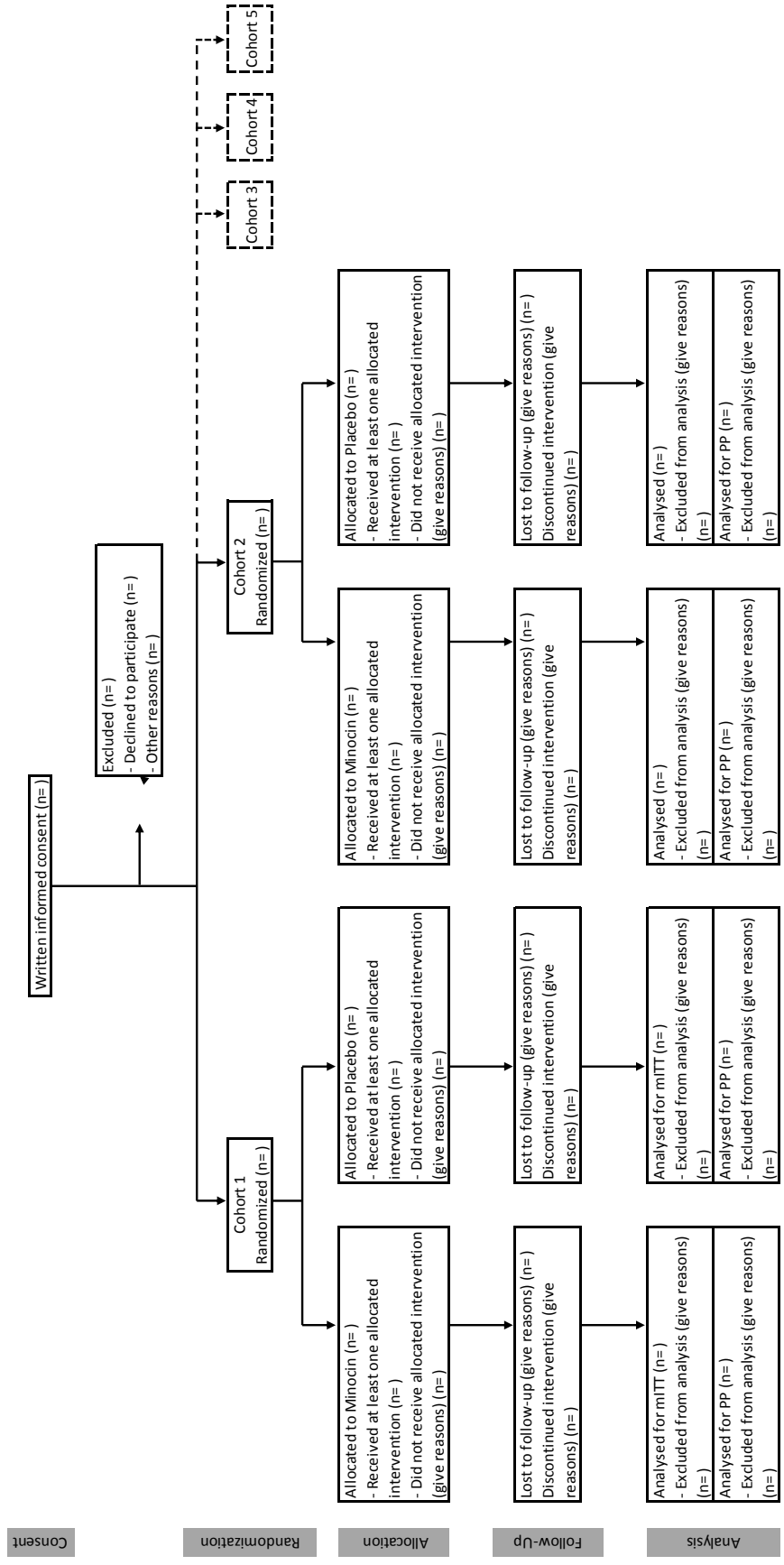


Figure 12-1: Flow diagramm

12.5 List of abbreviations

AE	Adverse Event
BMI	Body mass index
c%	Column percent
CS	Clinically significant
CTCC	Clinical Trial Centre Cologne
DMC	Data Monitoring Committee
ECG	Electrocardiography
IMSIE	Institute for Medical Statistics, Informatics and Epidemiology University of Cologne
ITT	Intent-To-Treat
max	Maximum
min	Minimum
mITT	modified Intent-To-Treat
MedDRA	Medical Dictionary for Regulatory Activities
PCS	Potentially clinically significant
PK	Pharmacokinetic
Q1	First quartile
Q3	Third quartile
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SD	Standard Deviation
SOP	Standard Operating Procedure
WHO	World Health Organization