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, Open-Label, Single-Dose Trial To Determine The Safety And Pharmacokinetics Of Minocin® (Minocycline) For Injection In Subjects With Renal Insufficiency
Study code:	Minocin 702 EudraCT No.: 2016-002247-41
Indication:	Healthy subjects and subjects with renal insufficiency
Investigational intervention:	Minocin® (minocycline) for Injection
Comparator:	None
Sponsor (or representative):	The Medicines Company 8 Sylvan Way Parsippany, NJ 07054
Financial support:	---
Protocol identification:	MDCO-MIN-16-03 v2.0, 30-NOV-16
Development phase:	Phase 1
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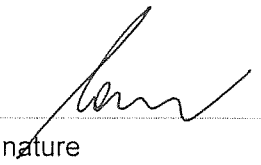
Approved by

Oliver Cornely, MD
Medical Lead

Köln, 13.6.17 — Place and date

 — Signature

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Trial Statistician

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Content

1 Background

This analysis plan details the analyses that will be performed for Minocin 702 Trial, a phase 1 study.

1.1 Trial objective

To evaluate the safety and tolerability of Minocin IV (Minocycline for Injection) in subjects with renal insufficiency and in subjects receiving hemodialysis (HD) therapy.
To assess the pharmacokinetics (PK) of Minocin IV in subjects with renal insufficiency and in subjects receiving HD therapy.

1.2 Trial design

This is a Phase 1, open label, single dose study to assess the safety, tolerability, and PK of intravenously administered Minocin IV in adults with varying degrees of renal insufficiency and in adult subjects receiving HD therapy as compared to subjects with normal renal function.
There will be at least eight subjects assigned to each of the following groups based on estimated glomerular filtration rate (eGFR) calculated at screening, using CKD-EPI 2009 for Groups 1-3, and using Cockroft-Gault for Group 4 (normal renal function). Group 5 is instead assigned by the requirement for HD therapy.

- Group 1: Mild renal insufficiency (eGFR 60-89 mL/min/1.73m^2)
- Group 2: Moderate renal insufficiency (eGFR 30 to < 60 mL/min/1.73m^2)
- Group 3: Severe renal insufficiency (eGFR < 30 mL/min/1.73m^2) not receiving HD therapy
- Group 4: Healthy subjects with normal renal function (eGFR ≥ 90 mL/min/1.73m^2)
- Group 5: Subjects with end stage renal disease (ESRD) receiving HD therapy 3 times a week for at least 3 months prior to Day 1

Subjects with mild, moderate, and severe renal insufficiency (Groups 1, 2, and 3, respectively) will be enrolled in the study concurrently.
Once the mild, moderate, and severe renal insufficiency groups are fully enrolled, healthy subjects (Group 4) will be matched to the pooled mean values of age (± 10 years), body mass index (BMI; ± 20%), and gender.
Group 5 will be enrolled after completion of Groups 1 - 4. It will include subjects who require HD therapy 3 times a week for at least 3 months prior to Day 1. Subjects in Group 5 will receive Minocin administered intravenously twice over the course of the study, once before HD therapy (Period 1) and once after (Period 2). Periods 1 and 2 do not have to occur in sequence for any given subject. A Washout Period will occur between the 2 Periods

regardless of the sequence of the Periods. The Washout Period will be scheduled to ensure that study drug administration is at least 6 days apart and no more than 14 days apart.

The planned length of participation in the study for each subject with mild, moderate, or severe renal insufficiency and normal renal function (excluding screening) is approximately 5 days from check-in on Day -1 (Day -1 procedures can also be completed pre-dose on Day 1 at the site's discretion) through completion of the Day 4 post-dose procedures. Subjects will remain within the clinic for the duration of the single dose period until completion of the post-dose procedures on Day 4. Subjects will receive a follow-up phone call on Day 6 (with a window of + ≤2 days). The total duration of participation, excluding screening period and including follow-up call, for each subject will be approximately 7-9 days.

Subjects with HD will participate in two 5 day periods; Periods 1 and 2, each from check-in on Day -1 (Day -1 procedures can also be completed pre-dose on Day 1 at the site's discretion) through completion of the Day 4 post-dose procedures. During each period, subjects will be confined to the clinic during Day -1 through Day 4. To separate Periods 1 and 2, a Washout Period will be scheduled to ensure that study drug administration is at least 6 days apart and no more than 14 days apart. Subjects will receive a follow-up phone call on Day 6 (with a window of + ≤ 2 days) after their second dosing. The total duration of participation, excluding screening period, for each subject will be approximately 12-23 days.

Subjects that discontinue the study early are required to complete Early Termination procedures.

Safety will be assessed throughout the study, and serial blood samples will be collected for the safety and PK assessment of Minocin IV.

The schedule of events/assessments is given in Figure 1-1 and Figure 1-2 and a schematic diagram of trial design is shown in Figure 1-3.

The study is planned to take place over approximately 24 weeks depending on rate of enrollment.

Figure 1-1 Schedule of events/assessments for groups 1 - 4

STUDY DAY►	SCREEN (<28)	-1[1]	1	1	1	1	1	1	1	2	2	3	4 Final Visit	6 (+≤2) Follow-Up Call
STUDY HOUR ►			0	1	2	4	8	12	18	24	36	48	72	
EVENT▼														
Informed Consent	X													
Med. History / Demographics	X	X												
Conmed Review	X	X----------												
BMI	X	X												
PE / Charlson Comorbidity Index	X	X											X	
RR/BP/HR/Temperature	X	X	X	X		X				X		X	X	
ECG (12-lead)[2]	X	X	X			X				X			X	
Hem/Coag/Chem/UA[3]	X	X											X	
Urine Drug/Alcohol Screen	X	X												
Pregnancy Test[4]	X	X	X										X	
Adverse Events			X----------											
CrCl / 24 h urine collection			X											
PK Blood Collection			X	X	X	X	X	X	X	X	X	X	X	
Inclusion/Exclusion Criteria	X	X												
Dose (1h infusion)			X											

Abbreviations: Conmed=concurrent medications; BMI=Body Mass Index; RR=respiratory rate; BP=blood pressure; HR=heart rate; ECG=electrocardiogram; Hem=hematology; Coag=coagulation; Chem=chemistry; UA=urinalysis; CrCl=creatinine clearance; PE=physical examination; PK=pharmacokinetics; h=hour

[1] Day -1 procedures, with the exception of triplicate ECG and assessment of Adverse Events, only need to be repeated if screening was >3 days prior to Day 1. Day -1 procedures can also be completed pre-dose on Day 1 at the site's discretion.

[2] On Day -1, ECGs should be performed in triplicate and may be repeated if necessary at the Investigator or Sponsor's discretion.

[3] Serum Creatinine from Screening Chemistry will be used for calculation of CrCl for renal function determination. Cockcroft-Gault will be used for Group 4 (normal renal function) and CKD-EPI 2009 for Groups 1-3 (renal insufficiency).

[4] Serum pregnancy should be performed during screening and Day -1. Urine pregnancy test should be performed before dosing on Day 1.

		Period 1[1]				Washout Period[2]	Period 2 [1]				
STUDY DAY►	Screening Period (<28)	-1	1	2	3 (48 h)	4 (72 h)	(Duration 1-9 days)	-1	1	2	3 (48 h)	4 (72 h)	6 (+≤2) Follow-up Call
Informed Consent	X												
Medical History / Demographics	X	X						X					
Conmed Review	X	X----------|					X----------|				X
BMI	X	X						X					
PE / Charlson Comorbidity Index	X	X				X		X				X	
RR/BP/HR/Temperature[3]	X	X	X	X	X	X		X	X	X	X	X	
ECG (12-lead) [4]	X	X	X	X		X[10]		X	X	X		X[10]	
Hem/Coag/Chem	X	X				X[10]		X				X[10]	
Drug Screen	X	X						X					
Alcohol Screen	X	X						X					
Pregnancy Test[5]	X	(X)				X[10]		(X)				X[10]	
Adverse Events[6]		(X)	X----------|				(X)	X----------|			X
Hemodialysis[7]			X		(X)	(X)			X		(X)	(X)	
Hourly dialysate aliquots[7]			X						X				
PK Blood Collection[8]			X	X	X	X			X	X	X	X	
Inclusion/Exclusion Criteria	X	X[9]						X[9]					
Dose (1h infusion) – prior to dialysis (Period 1)			X										
Dose (1h infusion) – after dialysis (Period 2)									X				

[1] Periods 1 and 2 do not have to occur in sequence, but washout period is required regardless of order. Procedures denoted by brackets "(X)" vary by period order / subject (see footnotes for each).
[2] The Washout Period will occur between the Periods 1 and 2, and will be scheduled to ensure that study drug administration is 6-14 days apart. Any AEs will be reviewed at the time of 2nd check-in.
[3] Vital signs, including RR/BP/HR/Temperature, will be taken at: pre-dose, 1, 4, 24, 48, and 72 hours after the start of dosing for both Periods 1 and 2.
[4] ECG (12-lead) will be taken at: Day -1 (in triplicate at Day -1 of the 1st period conducted), then at pre-dose, 4, 24, and 72* hours after the start of dosing for both Periods 1 and 2. (*See footnote 10)
[5] Serum pregnancy should be performed during screening, the first Day -1 (whichever period is conducted first), and at the final Day 4 visit (whichever period is completed last).
[6] Adverse events will be reviewed from Day 1 time of dosing through to the Day 6(+ ≤2] Follow Up Call. AEs during the washout should be recorded at the second Day -1, upon subject's return to site.
[7] HD will be done Day 1 pre or post dosing, depending on Period (1 or 2), then at Day 3 OR 4, then as per subject's usual schedule throughout the washout period; In Period 1, Day 1 a pre-spent dialysate sample is collected prior to initiating HD, then hourly dialysate samples (with aliquots) through the end of HD; In Period 2, Day 1 only a pre-spent dialysate sample is required.
[8] Plasma samples will be collected for PK assessments at: 0, 1, 2, 4, 8, 12, 18, 24, 36, 48, 72 hours after the start of dosing for both Periods 1 and 2.
[9] Inclusion/Exclusion Criteria should be checked at day -1 of whichever period is conducted first (Day -1 before the subject's first day of dosing).
[10] Complete these assessments only if Day 4 is the final, end-of-study visit for a subject. Because Periods 1 and 2 do not have to occur in sequence, the Day 4 of either period may be a subject's final in-patient visit. For the first period (prior to washout), do not complete these procedures. For an early termination complete all of these procedures.

Figure 1-2: Schedule of events/assessments for group 5

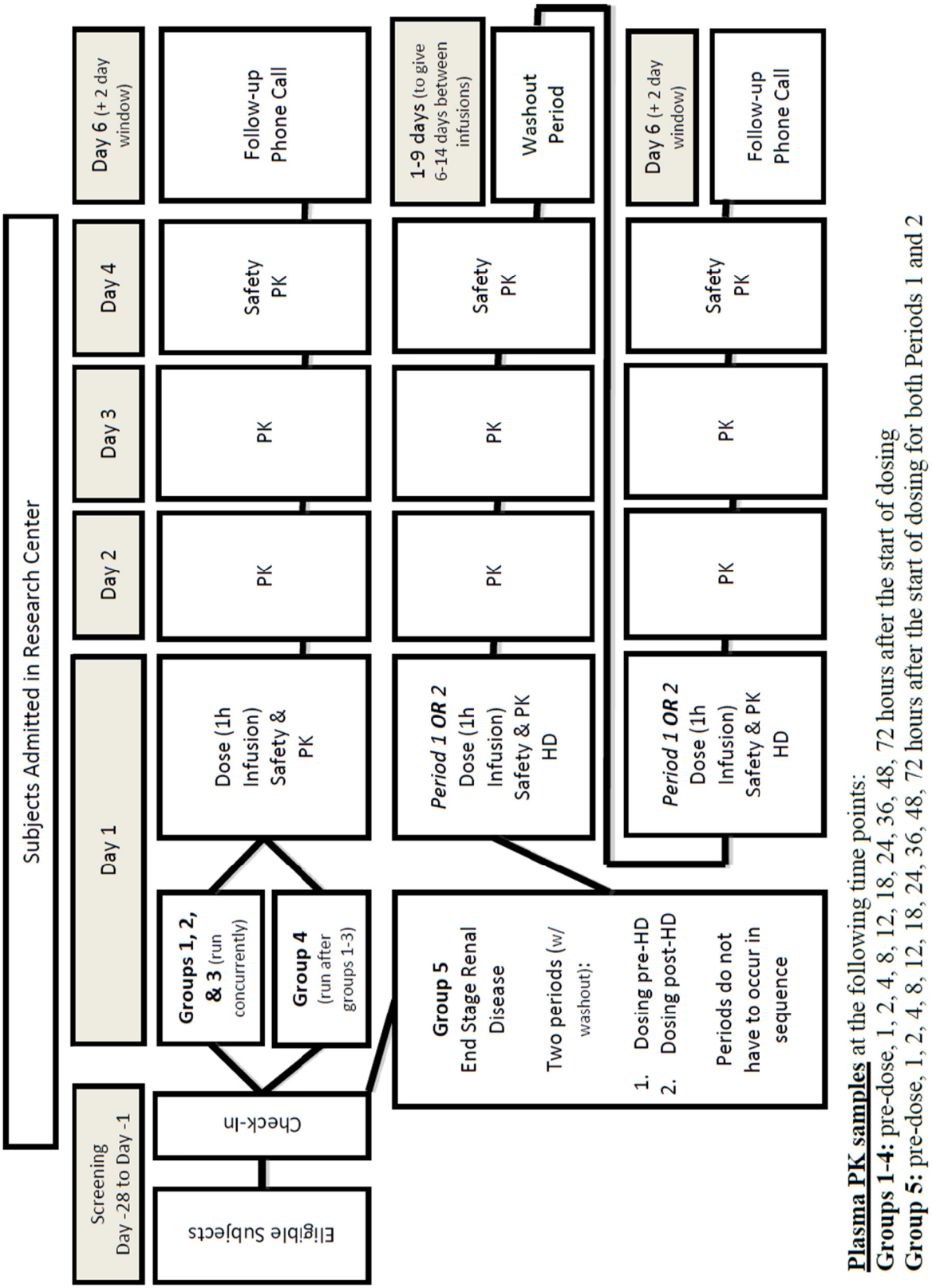

Figure 1-3: Schematic diagram of trial design

1.3 Timing of Analyses

After completion of the last visit of the last subject, when (at best) 40 subjects passed the complete trial, 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).

1.4 Sample Size

This sample size was selected based on previous experience attempting to minimize the number of subjects on study drug while obtaining sufficient PK data from different subjects to produce a good estimate of exposure. It has been determined adequate to meet the study objectives. Eight subjects per group are planned, hence n = 40 subjects in total.

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 enrolled into the trial.

Modified Intent-to-Treat (mITT) Population
All ITT subjects who receive at least one dose of study drug.

Pharmacokinetics (PK) Population
All subjects who have any valid samples measured for study drug levels. This will be used for PK analysis.

Per-Protocol (PP) Population
All mITT subjects who received their assigned study drug according to protocol. For Group 5, the time between dosing and HD therapy during Period 2 must not exceed 3h. The PP population will be the supportive population for the analyses.

Safety Population
The safety set will be the mITT population.

2.2 Application

Decision on the analysis populations is made prior to data lock.

The safety population and the PP 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. Drop-outs will be listed with group, time and reason (see 12.3, 2.).

3 Trial centres

This clinical trial will be carried out at a single German study site –
University Hospital Cologne
Department II for Internal Medicine
Kerpener Str. 62
50937 Koeln, Germany
The planned sample size is 8 subjects per cohort for all 5 groups.

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 (Period 1).
The following demographic variables will be analyzed:

- Age [years]
- Sex [M, F]
- Race [White, Black, Asian, Hawaii, Indian, Other]
- Estimated glomerular filtration rate (eGFR)

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]
- Charlson Comorbidity Index
  - Myocardial infarct [Yes, No]
  - Congestive heart failure [Yes, No]
  - Peripheral vascular disease [Yes, No]
  - Dementia [Yes, No]
  - Chronic pulmonary disease [Yes, No]
  - Connective tissue disease [Yes, No]
  - Ulcer disease [Yes, No]
  - Mild liver disease [Yes, No]
  - Diabetes [Yes, No]
  - Hemiplegia [Yes, No]
  - Moderate or severe renal disease [Yes, No]
  - Diabetes with end organ damage [Yes, No]
  - Any tumor [Yes, No]
  - Leukemia [Yes, No]
  - Lymphoma [Yes, No]
  - Moderate or severe liver disease [Yes, No]
  - Metastatic solid tumor [Yes, No]
  - AIDS [Yes, No]
  - Sum of score
- 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]

  For each ECG measurement (1., 2. and 3. measurement):
  - ECG Mean Ventricular Rate [bpm]
  - PR Interval [msec]

- QRS Duration [msec]
- QT Interval, uncorrected [msec]
- QT Interval, corrected [msec]

- Laboratory Tests
  - 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 [%; clinical significant]
    - Basophils [%; clinical significant]
    - Monocytes [%; clinical significant]
    - Lymphocytes [%; clinical significant]
    - Red blood cell counts [x10^12/l; clinical significant]
    - Platelet count [x10^12/l; clinical significant]
    - Prothrombin Intl. Normalized Ratio (INR) [%; clinical significant]
    - Activated partial thromboplastin time (aPTT) [sec; clinical significant]
  - Safety Lab: Chemistry
    - Urea [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]
    - Asparate 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]
    - 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 [negative, positive; clinical significant]
        - Additional microscopic exam [yes, no; clinical significant]
- Alcohol and drug screening
    - Alcohol [negative, positive]
    - Drug [negative, positive]
- Pregnancy test
    - Pregnancy test not done [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:

- 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]
    - 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 4 Period 1, Day 4 Period 2 (only Group 5))
  - 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 [%; clinical significant]
    - Basophils [%; clinical significant]
    - Monocytes [%; clinical significant]
    - Lymphocytes [%; clinical significant]
    - Red blood cell counts [x10^12/l; clinical significant]
    - Platelet count [x10^12/l; clinical significant]
    - Prothrombin Intl. Normalized Ratio (INR) [%; clinical significant]
    - Activated partial thromboplastin time (aPTT) [sec; clinical significant]
  - Safety Lab: Chemistry
    - Urea [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]
    - Asparate 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]
        - 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 [negative, positive; clinical significant]
        - Additional microscopic exam [yes, no; clinical significant]
- Vital Signs (Day 1 (1h, 4h), Day 2 - 4, Period 1 and Period 2 (only Group 5))
    - Systolic Blood Pressure [mmHg]
    - Diastolic Blood Pressure [mmHg]
    - Pulse rate [beats/min]
    - Respiratory rate [breaths/min]
    - Temperature [°C]
- ECGs (Day 1 (4h), Day 2, Day 4, Period 1 and Period 2 (only Group 5))
    - ECG result [normal, clinically insignificant abnormality, clinically significant abnormality, not assessable]

  For each ECG measurement (1., 2. and 3. measurement):
    - ECG Mean Ventricular Rate [bpm]
    - PR Interval [msec]
    - QRS Duration [msec]
    - QT Interval, uncorrected [msec]
    - QT Interval, corrected [msec]
- Physical Examination (Day 4, Period 1 and Period 2 (only Group 5))
    - Height [cm]
    - Weight [kg]
    - BMI [kg/m^2]
    - Skin [normal, abnormal, not done]
    - Head [normal, abnormal, not done]
    - Ears, eyes, nose and throat [normal, abnormal, not done]
    - Respiratory system [normal, abnormal, not done]

  - Cardiovascular system [normal, abnormal, not done]
  - Gastrointestinal system [normal, abnormal, not done]
  - Neurological condition [normal, abnormal, not done]
  - Blood and lymphatic system [normal, abnormal, not done]
  - Musculoskeletal system [normal, abnormal, not done]
  - Other [normal, abnormal, not done]
- Charlson Comorbidity Index (Day 4, Period 1 and Period 2 (only Group 5))
  - Sum of score
- Creatinine clearance (Day 1, Period 1 (only Group 1 - 4))
  - Result [Unit]
- HD therapy and dialysate sampling (only Group 5: Day 1, Period 1 and 2)
  - Dialysis performed [yes, no]
  - Start date of dialysis [DD/MM/YYYY]
  - Start time of dialysis [HH:MM]
  - End of dialysis [HH:MM]
  - Planned time of sampling [HH:MM]
  - Time of sampling [HH:MM]
  - Sampling not done [yes, no]
- Medication error (Day 1, Period 1 and Period 2 (only Group 5))
  - Are there any medication errors [yes, no]
  - Kind of medication error [wrong study drug, overdose, underdose, wrong route of administration, abuse of medicinal product, misuse, wrong patient, other]
  - Medication error associated with AE/SAE [yes, no]

4.3 PK Variables

Plasma samples for PK analysis will be collected relative to dosing. The actual sampling times will be used in the parameter calculations and PK parameters will be derived using standard non-compartmental methods in Phoenix WinNonLin® version 6.2 or higher.

The following Plasma PK parameters will be assessed from the plasma concentration of single dose of Minocin IV will be derived.

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

If required then additional parameters will be derived and included in CSR. The maximum plasma concentration (C_{max}), the time of maximum concentration (t_{max}) will be determined by inspection of the concentration-time profiles. The AUC_{0-t} will be calculated using the linear trapezoidal rule. Where appropriate, the AUC_{0-t} will be extrapolated to infinity using λz to obtain AUC_{0-inf}.

5 Handling of missing values and outliers

5.1 Missing values

Missing values will not be imputed. Instead, subjects withdrawn for reasons other than drug related AEs with fewer than 4 PK samples within 24 hours of dosing (at either period for Group 5 subjects) will be replaced. Subjects who drop-out/are withdrawn before taking any study drug will be replaced.

If subjects drop-out after dosing but before end of study all data collected up until the time of subject withdrawal is to be entered into the electronic case report form (eCRF). In addition, every attempt should be made to collect follow-up information except for those subjects who specifically withdraw consent for release of such information. All procedures and laboratory specimens or tests requested for evaluation following administration of the study drug should be carried out when possible.

5.2 Outliers

A special outlier handling is not done.

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 and with given written informed consent).

Definition of mITT population: All ITT subjects with number of administered infusions ≥ 1.
Definition of PP population: All mITT subjects with number of administered infusions = 1 (Group 1 - 4) or 2 (Group 5), number of administered doses per protocol = 1 (Group 1 - 4) or 2 (Group 5). For Group 5: Time between dose and HD therapy <= 3h and the time difference between the two doses is 6 and 14 days.
Definition of PK population: All subjects with at least one administered infusion and any valid PK blood or urine sampling.
Definition of safety population: All subjects of the mITT population.

Absolute and relative frequencies of patients per group 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) will serve as the baseline value. For Group 5 the baseline for Period 2 will be updated after Washout Period with the last measurement before second dosing (Day -1 or Day 1 of Period 2).
Demographic and baseline variables will be summarized descriptively by group (for Group 5 both baselines, at period 1 and 2 will be described) for the safety and PP population. For demographics, the total of groups 1 to 5 at the baseline of Period 1 will be given.

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 group and patients 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 group.

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, 6.).

6.5 Exposition to treatment/Compliance

Number of subjects in the ITT population with number of administered infusions = 0 (Group 1 to 4) and = 0/1 (Group 5) will be calculated per group.

Listing of drop-outs will be given (see 2.3 and 12.3, 2.).

6.6 Analysis of safety and tolerability

Variables for the analysis of safety and tolerability will be evaluated in the safety population as well as in the PP population.
Tables and listings presented by group will always list ‘Group 5 dose before HD’ and ‘Group 5 dose after HD’ separately.

6.6.1 Adverse events/Serious adverse events

All AEs occurring from the time when informed consent is obtained at screening up to the last follow-up visit (phone call on Day 6) are listed by subject with group, AE number, AE Term, preferred term (MedDRA dictionary), primary system-organ class, start date, start time, end date, end time, ongoing at study end, severity, causality, therapy, therapy specification, outcome and seriousness (see 12.3, 3.).
Additionally, all SAEs will be listed by subject with 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, 4.).
Listing of AE and SAE will be done separated by pre- and post-treatment. For Group 5 in Period 2 only Day -1 and Day 1 (before dosing) are considered as pre-treatment.
In the following section a treatment emergent adverse event (TEAE) defines an AE that occurred or worsened after the start of the treatment.

The total number (percentage) of TEAEs will be tabulated by 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 TEAEs will be tabulated by group and classified by:

1. any TEAE, related TEAE, unrelated TEAE, 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 TEAEs or SAEs, related and not related to study drug, then the subject is counted as related (‘causality’ = yes). If a subject has multiple TEAEs/SAEs with

the same preferred term, it is counted only once. Likewise, if a subject has multiple TEAEs with the same preferred term but different severities, the maximal severity is used and the subject is counted only once.

6.6.2 Laboratory tests (Hematology, Chemistry, Urinalysis)

Laboratory variables will be summarized descriptively by group, including mean change and mean percent change 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 Day 4, of each parameter will be given by group. For Group 1 - 4 one table per variable is given for baseline vs. Day 4. For Group 5, comparisons of baseline vs. Day 4 for both periods, dose before and after HD therapy, are given (see Table 12-8).

6.6.3 Vital signs

Vital signs will be summarized descriptively by group, including mean change and mean percent change from (period-corresponding) 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 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 group for each scheduled time point. Additionally, a shift table (see Table 12-8) will be presented for ECGs results for each group (Group 1 - 4), comparing baseline to Day 4. For Group 5, comparisons of baseline vs. Day 4 for both periods, dose before and after HD therapy, are given.

6.6.5 Physical Examination

Physical examination variables will be summarized descriptively by group for each scheduled time point.
Furthermore, for all variables assessed as normal/abnormal/not done, normal-abnormal shift tables (see Table 12-8) will be presented by group. For Group 1 - 4 one table per variable is given baseline vs. Day 4. For Group 5, comparisons of baseline vs. Day 4 for both periods, dose before and after HD therapy, are given.

6.6.6 Charlson Comorbidity Index

Sum of score will be summarized descriptively by group for each scheduled time point with additional rows for mean change and mean percent change from (period-corresponding) baseline (see Table 12-7).

6.6.7 Creatinine clearance

Result of creatinine clearance will be summarized descriptively by group (only Group 1 - 4).

6.6.8 HD therapy and dialysate sampling

Number of performed dialysis and number of dialysate samples are summarized descriptively for Group 5.

6.6.9 Medication error

Occurrence of medication errors, kind of medication errors and associations with AE/SAE will be summarized descriptively. Additionally medication errors will be listed with kind of medication error, other error specification, medication error associated with AE/SAE and referring AE No (see 12.3, 5.).

6.7 Pharmacokinetic analyses

Pharmacokinetic variables will be evaluated in the PK population.

6.8 Planned subgroup analyses

Analysis of PK and analysis of AEs will be performed stratified by sex.

6.9 Interim analyses

Not applicable.

7 Deviations from the protocol

Additional to all pre-specified analyses in the study protocol this SAP contains further sections with specifications concerning the analysis of Physical Examination, Charlson Comorbidity Index, Creatinine Clearance, HD therapy and dialysate sampling and Medication error.

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-16-03, Version 2.0, final 30-NOV-16.

12 Appendices

12.1 Reference ranges of laboratory parameters

- Hemoglobin (male): 13.5 – 18 g/dl
- Hemoglobin (female): 12 – 16 g/dl
- Hematocrit (male): 42 – 50 %
- Hematocrit (female): 36 – 45 %
- White blood cell count: 4.4 – 11.3 x10^9/l
- Neutrophils abs.: 2 – 7.5 x10^9/l
- Neutrophils: 53 – 75 %
- Eosinophils: 2 – 4 %
- Basophils: 0 – 2 %
- Monocytes: 25 – 40 %
- Lymphocytes: 2 – 14 %
- Red blood cell count (male): 4.5 – 5.9 x10^12/l
- Red blood cell count (female): 4 – 5.2 x10^12/l
- Platelet count: 150 – 400 x10^9/l
- Prothrombin Intl. Normalized Ratio (INR): 2 – 4.5
- Activated partial thromboplastin time (aPTT): 22 – 36 sec
- Urea: 0 – 50 mg/dl
- Serum Creatinine (male): 0.5 – 1.1 mg/dl
- Serum Creatinine (female): 0.5 – 0.9 mg/dl
- Total Bilirubin: 0 – 1.2 mg/dl
- Direct Bilirubin: 0 – 0.3 µmol/L
- Alkaline phosphatase (male): 40 – 130 U/L
- Alkaline phosphatase (female): 35 – 105 U/L
- Asparate transaminase (male): 0 – 50 U/L
- Asparate transaminase (female): 0 – 35 U/L
- Alanine transaminase (male): 0 – 50 U/L
- Alanine transaminase (female): 0 – 35 U/L
- Albumin: 35 – 52 g/L
- Total protein: 66 – 87 g/L
- Glucose: 74 – 109 mg/dL
- Calcium (age: 18 – 60 years): 2.04 – 2.59 mmol/L
- Calcium (age: 60 – 90 years): 1.93 – 2.60 mmol/L
- Chloride: 97 – 108 mmol/L

- Sodium: 135 – 145 mmol/L
- Magnesium: 0.7 – 1.1 mmol/L
- Potassium: 3.6 – 4.8 mmol/L
- Uric acid (male): 3.4 – 7.0 mg/dl
- Uric acid (female): 2.4 – 5.7 mg/dl
- Lactate dehydrogenase: 0 – 250 U/L
- Phosphorus: 0.81 – 1.45 mmol/L
- pH: 5 - 8
- Specific gravity: 1.010 - 1.025 g/L

Physical examination results are assessed as normal/abnormal and (if abnormal) clinically significant yes/no in the eCRF.

12.2 Planned tables

All tables with stratification by group follow the group order Group 4, 1, 2, 3, Group 5 dose before HD, Group 5 dose after HD. For simplification the following presentation contains example groups ('X', 'Y' and 'Z').

Table 12-1 Table of quantitative variables.

		Group X (n =)	Group Y (n =)	Group Z (n =)	Total (n =)
Quantitative Variable 1	n				
	mean				
	SD				
	median				
	Q1				
	Q3				
	min				
	max				
Quantitative Variable 2	n				
	mean				
	SD				
	median				
	Q1				
	Q3				
	min				
	max				
…					

Table 12-2 Table of qualitative variables.

		Group X (n =)	Group Y (n =)	Group Z (n =)	Total (n =)
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.

		Group X	Group Y	Group Z	Total
System-organ class	**SOC 1**	n (%)	…		
	SOC 2	…			
	…				
Preferred term	**Term 1**				
	Term 2				
	…				
Severity	**Grade 1**				
	…				
	Grade 5				
Causality	**Reasonable possibility**				
	No reasonable possibility				
Total					

Table 12-4 AE tabulation (version 1).

		Group X	Group Y	Group Z	Total
AE	**any**	n (%)	…		
	related	…			
	unrelated				
SAE	**any**				
	related				
	unrelated				

Table 12-5 AE tabulation (version 2).

		Group X	Group Y	Group Z	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	Group X	Group Y	Group Z	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 Shift table for quantitative variables (mean (percent) change from (period-corresponding) baseline).

		Group X (n =)	Group Y (n =)	Group Z (n =)	Total (n =)
Quantitative Variable 1	n				
	mean				
	SD				
	median				
	Q1				
	Q3				
	min				
	max				
	mean change				
	mean % change				
Quantitative Variable 2	n				
	mean				
	SD				
	median				
	Q1				
	Q3				
	min				
	max				
	mean change				
	mean % change				
…					

Table 12-8 Shift table for qualitative variables.

		Baseline, Period x			
		Category 1	**Category 2**	**…**	**Total**
Day 4, Period x	**Category 1**	n (%)	…		
	Category 2	…			
	…				
	Total				

12.3 Planned listings

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

Group	ID	Medication (tradename)	WHO drug name	Indication	Total daily dose	Unit	Route of administration
xx	xx	xxxxx	xxxxx	xxxxx	xxx	xx	xxxxx

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

2. Drop-outs with group, time, reason (Day 1 - Day 6, Period 1/2) (see 2.3, 0)

ID	Group	Date	Time	Reason
xx	xx	dd.mm.yyyy	hh:mm	xxxxx

3. All AEs 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 and group (see 6.6.1)

ID	Group	AE No.	AE Term	Preferred term (MedDRA)	System-organ class	Start date	Start time
xx	xx	xx	xxxxx	xxxxx	xxxxx	dd.mm.yyyy	hh:mm

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

Therapy specification	Outcome	Seriousness	CRF comment
xxxxx	xx	x	xxxxx

4. 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 and group (see 6.6.1)

ID	Group	SAE No.	AE No.	Preferred term (MedDRA)	System-organ class	Date AE turned serious	Time AE turned serious
xx	xx	xx	xx	xxxxx	xxxxx	dd.mm.yyyy	hh:mm

SAE diagnosis	Results in death	Life-threatening	Results in significant damage	Hospitalization
xxxxx	x	x	x	x

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

5. Medication error (see 6.6.9)

ID	Group	Are there any medication errors	Kind of medication error	Other error specification	Medication error associated with AE/SAE?	Referring AE No.
xx	xx	x	xxxxx	xxxxx	x	xx

6. Study Drug Administration (see 6.4)

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

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

12.4 Planned graphics

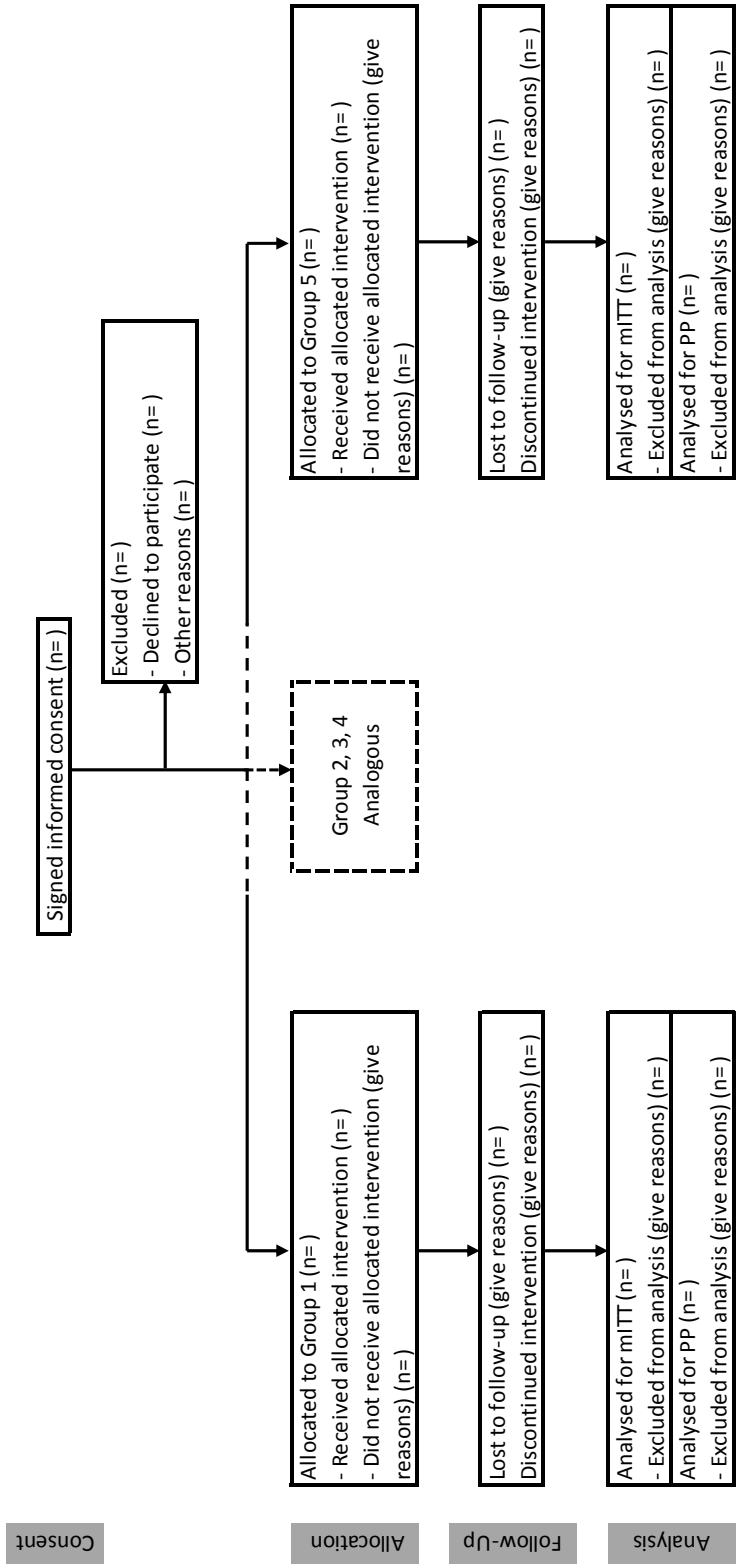

Figure 12-1 Flow chart.

12.5 List of abbreviations

AE	Adverse Event
BMI	Body mass index
BR	Breathing rate
c%	Column percent
CS	Clinically significant
CSR	Clinical Study Report
CTCC	Clinical Trial Centre Cologne
DMC	Data Monitoring Committee
ECG	Electrocardiography
HD	Hemodialysis
HR	Heart rate
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
NCS	Not clinically significant
PCS	Potentially clinically significant
PK	Pharmacokinetic
PP	Per-Protocol
Q1	First quartile
Q3	Third quartile
RR	Blood pressure (Scipione Riva-Rocci)
SAE	Serious Adverse Event
SADR	Serious Adverse Drug Reaction
SAP	Statistical Analysis Plan
SD	Standard Deviation
SOP	Standard Operating Procedure
TEAE	Treatment Emergent Adverse Event
WHO	World Health Organization