Statistical Analysis Plan

Protocol Number: MCI-186-J25

A Randomized, Single-Blind, Placebo-Controlled, Three-Way Crossover Study to Evaluate the Effect of MCI-186 at Therapeutic and Supra-Therapeutic Doses on the QT/QTc Interval in Healthy Subjects

Version Number: 2.0
Date: January 25, 2019
NCT number: NCT04029090

STATISTICAL ANALYSIS PLAN

Protocol No.	MCI-186-J25
Protocol Title	A Randomized, Single-Blind, Placebo-Controlled, Three-Way Crossover Study to Evaluate the Effect of MCI-186 at Therapeutic and Supra-Therapeutic Doses on the QT/QTc Interval in Healthy Subjects
Version History	Version 1.0 First approved version / 16 January, 2019 Version 2.0 Final approved version / 25 January, 2019
Author	[redacted]

APPROVAL FORM

The approval signatories below have reviewed this Statistical Analysis Plan (SAP) and agreed on the planned analyses defined in this document.

MTPC Statistics Approver		[MTPC-STATH]
[redacted]	Date:	Signature:
MTPC Pharmacokinetics Approver		[MTPC-STATH]
[redacted]	Date:	Signature:

TABLE OF CONTENTS

Abbreviations

AE	:	adverse event
BLQ	:	below limit of quantification
BMI	:	body mass index
CV	:	coefficient of variation
DBL	:	database lock
DP	:	decimal places
ECG	:	electrocardiogram
IAO	:	International agreed order
IMP	:	investigational medicinal product
LLOQ	:	lower limit of quantification
MedDRA:		Medical Dictionary for Regulatory Activities
PK	:	pharmacokinetics
PT	:	preferred term
QC	:	quality control
SAP	:	statistical analysis plan
SAE	:	serious adverse event
SD	:	standard deviation
SOC	:	system organ class
TEAE	:	treatment emergent adverse event
WHO-DD:		World Health Organization Drug Dictionary

List of PK Parameters		
Parameters	**Unit**	**Definitions**
α	1/h	Elimination rate constant of the α-phase
$AUC_{0\text{-last}}$	h*ng/mL	Area under the concentration-time curve from time zero to the last quantifiable concentration time point
$AUC_{0\text{-}24h}$	h*ng/mL	Area under the concentration-time curve from time zero to 24h
$AUC_{0\text{-}\infty}$	h*ng/mL	Area under the concentration-time curve from time zero to infinity
$AUC\%_{ex}$	%	Area under the plasma concentration-time curve extrapolated from the last quantifiable concentration time point to infinity in % of the total $AUC_{0\text{-}\infty}$
β	1/h	Elimination rate constant of the β-phase
CL	L/h	Total clearance
C_{max}	ng/mL	Maximum plasma concentration
γ	1/h	Elimination rate constant of the γ-phase
k_{21}	1/h	Transfer rate constant from peripheral (2) to central (1) compartment
k_{31}	1/h	Transfer rate constant from peripheral (3) to central (1) compartment
k_{el}	1/h	Elimination rate constant from the central compartment in compartmental analysis
Lower limited of λ_z	h	Lower data point used for the estimation of λ_z
MRT	h	Mean residence time
Number of λ_z points	-	Number of data point used for the estimation of λ_z
A	ng/mL	Zero-time intercept for α-phase
B	ng/mL	Zero-time intercept for β-phase
C	ng/mL	Zero-time intercept for γ-phase
λ_z	1/h	Elimination rate constant in non-compartmental analysis
$t_{1/2}$	h	Terminal elimination half-life in plasma concentration-time course
$t_{1/2\alpha}$	h	Half-life at α-phase
$t_{1/2\beta}$	h	Half-life at β-phase
$t_{1/2\gamma}$	h	Half-life at γ-phase
t_{max}	h	Time of maximum plasma concentration after administration

List of PK Parameters		
Upper limited of λ_z	h	Upper data point used for the estimation of λ_z
V_1	L	Volume of the central compartment
V_{SS}	L	Volume of distribution at Steady State
V_z	L	Volume of distribution during terminal phase

1. INTRODUCTION

This statistical analysis plan (SAP) is based on the final protocol dated 29 August 2018. The plan covers statistical analysis plan, tabulations and listings of to evaluate the effect of MCI-186 at therapeutic and supra-therapeutic doses on the QT/QTc interval in healthy subjects. Holter ECG analysis with the related section of 2 will be performed based on a separate ECG SAP.

The SAP is prepared by [redacted] The statistical analyses and production of the outputs described in the SAP will be conducted and QC checked by [redacted] Data Science Department, using SAS® Version 9.2 or higher. The final analyses and outputs will be approved by Mitsubishi Tanabe Pharma Corporation Data Science Department.

1.1 Study Design

This is a Phase I, randomized, single-blind, placebo-controlled, three-way crossover single-center study.

Up to 27 healthy male volunteers, aged 20 to 55 years who meet the study criteria will be allocated to 3 treatment sequences of 9 subjects each. In each sequence, subjects will receive a single I.V. dose of MCI-186 as therapeutic or supra-therapeutic dose, or a matching dose of placebo in a fasted state.

The study consists of the following 3 treatments and 3 sequences;

- Treatment A (therapeutic dose of MCI-186): A single dose of 60 mg MCI-186 over 60 min will be intravenously administered.
- Treatment B (supra-therapeutic dose of MCI-186): A single dose of 300 mg MCI-186 over 60 min will be intravenously administered.
- Treatment C (Placebo): A single dose of 0.9% w/v saline over 60 min will be intravenously administered.

Sequence	Period 1	Period 2	Period 3
1	A	C	B
2	B	A	C
3	C	B	A

1.2 Schedule of Study Procedures

Study assessments are summarized in the time and events schedule in Table 1

Table 1 Time and events schedule

Study Period	Screening	Confinement (Next IMP dosing must keep at least 72 hr and up to 9 days washout after starting the previous dosing)															Follow-up
Study Day	**-28 to -3**	**-1**	**1**													**2**	**5-9**
Time point in Relation to Start of Infusion			Pre-dose	0	0.5 h	1 h	75 min	90 min	105 min	2 h	3 h	4h	6h	8h	12h	24h	(only Period 3)
Informed consent	X																
Confinement[a]		<--	----	----	----	----	----	----	----	----	----	----	----	----	----	-->	
Outpatient	X																X
Demography & medical history	X																
Inclusion/exclusion criteria	X	X	X														
Randomization			X														
Physical examination	X	X[b]	X[b]			X[b]										X[b]	X
Weight	X	X														X	X
Height	X																
BMI	X	X															
Vital signs[c]	X	X	X			X										X	X
Routine safety 12-lead ECG[d]	X[e]	X	X			X										X	X
Continuous 12-lead Holter (PD ECGs extracted in triplicate)[f]			X[g]		X	X	X	X	X	X	X	X	X	X	X	X	
Drugs of abuse & alcohol test	X	X															
Hematology, biochemistry, coagulation & urinalysis	X	X														X	X
Serology	X																
IMP administration (infusion)				<--	----	-->											
PK sampling (blood)[h]			X		X	X	X	X	X	X	X	X	X	X	X	X	
Adverse events	<--	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	-->
Concomitant medications	<--	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	-->

a: Subjects will be admitted 3 times to the study center (on Day -1 in each period) and discharged in the morning of Day 2 in each period.
b: Abbreviated physical exam
c: Blood pressure, pulse rate and body temperature
d: Using conventional bedside equipment
e: Routine safety ECG in triplicate at Screening only
f: Mandatory rest from 10 minutes prior to until 5 minutes after each timepoint
g: Predose at -45, -30 and -15 minutes
h: No sooner than 5 minutes after associated PD ECG timepoint

2. STUDY OBJECTIVES AND ENDPOINTS

In this SAP, the analyses with underline are not planed.

2.1 Study Objectives

2.1.1 Primary objective

- To evaluate the effect of MCI-186 on the QT interval corrected for heart rate using Fridericia's formula (QTcF)

2.1.2 Secondary objectives

- To evaluate the PK profile of MCI-186
- To evaluate the effect of MCI-186 on other 12-lead ECG intervals
- To evaluate the safety and tolerability of MCI-186

2.1.3 Primary endpoint

- Relationship of change from baseline in QTcF (ΔQTcF) with placebo adjustment (ΔΔQTcF) and concentration of MCI-186

2.1.4 Secondary endpoints

- Assessment of HR, PR interval, QRS interval, and QTcF by timepoint
- Categorical outliers for QTcF interval (absolute value and change from baseline) and other 12-lead ECG parameters
- Incidence of abnormalities in ECG morphology
- PK parameters of MCI-186
- Incidence of adverse events (AEs) and serious adverse events (SAEs), vital signs, safety 12-lead ECG variables, laboratory tests and physical examination

2.1.5 Exploratory endpoints

Not applicable in the study.

3. PLANNED ANALYSES

This study was planned to evaluate the effect of MCI-186 at therapeutic and supra-therapeutic doses on the QT/QTc interval in healthy subjects.

The statistical analyses will be performed after database lock(DBL). Interim analysis will not be carried out.

4. ANALYSIS POPULATION(S)

The statistical analysis will be based on the following defined populations:

- Safety population includes all randomized subjects who received at least one dose of IMP.
- PK population includes all randomized subjects who received at least one dose of IMP and who have at least one post-dose value of plasma concentration without important Protocol deviations which may affect the PK of the IMP.

For cases that subjects were not adopted in the PK analysis set, all PK data will be rejected and no parameters will be calculated. Furthermore, for cases that subjects adopted in the PK analysis set and partial data not being adopted, PK parameters will be calculated from only the adopted data.

The acceptance or rejection of each analysis population will be treated based on the results of the data review meeting on [redacted] as follows.

Safety analysis set: No subject is excluded from analysis.

PK analysis set: No subject is excluded from analysis.

5. GENERAL CONSIDERATIONS

5.1 Subjects Composition

- Treatment A (therapeutic dose of MCI-186):
  A single dose of 60 mg MCI-186 over 60 min will be intravenously administered.

- Treatment B (supra-therapeutic dose of MCI-186):
  A single dose of 300 mg MCI-186 over 60 min will be intravenously administered.

- Treatment C (Placebo):
  A single dose of 0.9% w/v saline over 60 min will be intravenously administered.

5.2 Analysis Time Window for Visits

1) Analysis time windows for blood sampling for PK measurements are as follows.

Pre-dose	Before dosing
0.5 h after dosing	Scheduled time + 5 to 7 minutes
1 h after dosing	Following completion of dosing + 5 to 7 minutes
75 min, 90 min and 105 min after dosing	Scheduled time + 5 to 7 minutes
2 h, 3 h, 4 h, 6 h, 8 h, 12 h and 24 h after dosing	Scheduled time + 5 to 10 minutes

2) Analysis time windows for vital signs and safety 12-lead ECG are as follows.

Screening (Day -28 to Day -3)	Not specified
Hospitalization (Day -1)	Not specified
Before dosing (Day 1)	From the time of waking up until the time when Holter ECG rest was secured (45 minutes before starting administration)
1 h after dosing (Day 1)	Scheduled time ± 20 minutes
24 h after dosing (Day 2)	From wake-up to scheduled time + 1 hour (25 hours after start of administration)
Follow-up (Day 7 ± 2 days)*	Not specified

*: Period 3 only

3) Analysis time windows for physical examinations are as follows.

Screening (Day -28 to Day -3)	Not specified
Hospitalization (Day -1)	Not specified
Before dosing (Day 1)	From the time of waking up until the time when Holter ECG rest was secured (45 minutes before starting administration)
1 h after dosing (Day 1)	Scheduled time + 1 hour 30 minutes
24 h after dosing (Day 2)	From wake-up to scheduled time + 1 hour (25 hours after start of administration)
Follow-up (Day 7 ± 2 days)*	Not specified

*: Period 3 only

4) Analysis time windows for blood and urine sampling at the safety laboratory tests are as follows.

Screening (Day -28 to Day -3)	Not specified
Hospitalization (Day -1)	Not specified
24 h after dosing (Day 2)	Blood sampling: From getting up to before breakfast Urine collection: From getting up to before breakfast
Follow-up (Day 7 ± 2 days)*	Not specified

*: Period 3 only

5.3 Handling of data for pharmacokinetic assessments

The allowable ranges for the collection of blood samples for the measurement of the plasma drug concentrations are shown in Section 5.2. Only valid PK data will be included in the summary tables or figures. PK data that are considered "invalid" or "abnormal" will be flagged in the listing. The PK data handling will be confirmed during data review.

5.4 Number of Digits to Report

Statistical analysis variables, statistics to be calculated and number of digits to report are as follows.

Routine safety laboratory tests Physical examinations Routine safety 12-Lead ECG Demography, body weight, height, BMI	Mean, SD, median	Report to one extra digit plus the determined/specified digits
	Minimum, maximum	Report to the determined/specified digits
Pharmacokinetics	Mean, SD, minimum, median, maximum, geometric mean	Report to the determined/specified digits
General information	Number of subjects, number of valid observations, number of events, number of cases	Integer
	CV%, geometric CV%, Percentages (%)	To the first decimal place

5.5 Significance level and confidence level

No statistical testing and estimation will be performed.

5.6 Descriptive statistics values to calculate

Where appropriate, continuous variables will be summarized descriptively, using the number of observations, mean, SD, median, minimum, and maximum. Categorical variables will be summarized using frequency counts and percentages.

5.7 Derived variables

(1) Definition(s) of baseline(s)

The baseline of vital signs and Routine safety 12-lead ECG is the last valid assessment obtained on or before Day 1 prior to the administration of single-blind IMP by each treatment group (60 mg group, 300 mg group and placebo group).

The baseline of body weight, BMI, and clinical laboratory values will be the value obtained on Day -1.

(2) Age at informed consent

Age (years) = Year of informed consent - Year of birth

Subtract 1 from the age (years) calculated above, if [Month of informed consent < Month of birth] or [Month of informed consent = Month of birth AND Day of informed consent < Day of birth].

(3) BMI

BMI = Weight [kg] / (Height [m])^2

Height will be the value obtained at screening.

(4) Adverse events

The MedDRA version 21.0 will be used as a unified dictionary in the assessment of AEs.

(5) Adverse reactions

Adverse reactions are defined as AEs that are determined to have a "Reasonable Possibility" of causal relationship to the IMP.

(6) Prior and Concomitant Medications

All medication data will be coded according to the latest version of WHO-DD and Anatomical Therapeutic Chemistry (ATC) classification.

6. SAMPLE SIZE AND POWER CONSIDERATIONS

The sample size of 27 subjects of IMP per treatments is not based on a formal power calculation. However, referring to the recent publication[1], even if assuming that is at most 5 msec ΔΔQTcF in C_{max} of MCI-186 300 mg as supra-therapeutic dose, 24 subjects of each doses MCI-186 or placebo are considered to be adequate to meet the objectives of the study at power = 80% or higher. In addition, 24 subjects will provide at least 90% power of the upper bound in 90% confidence interval is less than 10 msec, assuming 0 msec ΔΔQTcF at C_{max} of MCI-186 60 mg and 300 mg based on simulation using previous studies including MCI-186-E02. Assuming a few drop-out, a total of 27 subjects are planned to be randomized in this study.

7. STATISTICAL METHODOLOGY

7.1 Disposition of Subjects

Disposition of subjects will be listed.

- Number and percent of subjects completed and discontinued protocol with its reason will be presented by each sequence (1: A-C-B, 2: B-A-C and 3: C-B-A).
- Subjects withdrawn will be summarized by reasons for discontinuation.

7.2 Demographic and Other Baseline Characteristics

Major demographic and other baseline characteristics will be listed.

For each analysis set, major demographic and other baseline characteristics will be summarized by each sequence (1: A-C-B, 2: B-A-C and 3: C-B-A) and overall. For categorical values, frequency and percentage will be reported. For continuous values, descriptive statistics values (number of subjects, mean, SD, minimum, median, and maximum) will be calculated.

Table. Variables related to demographic and other baseline characteristics

Category	Variable	Data format
Subject background	Sex (male, female): display without female	Binary
	Age at consent acquisition (years)	Continuous
	Race	Categorical
	Japanese, Non-Japanese	Binary
	Height (cm)	Continuous
	Body weight (kg) on Day -1	Continuous
	BMI on Day -1	Continuous
	Medical history	Binary

7.3 Medical History

All medical history data will be listed.

7.4 Prior and Concomitant Medications

All medication data will be listed.

Prior medication is any medication taken only in the two weeks prior to administration of IMP. Concomitant medication is defined as any medication, other than the IMP, which is taken from the start of IMP infusion to the Follow-up assessment, including prescription, herbal and over-the-counter medications.

Prior and Concomitant medications will be summarized by each treatment group (60 mg group, 300 mg group and placebo group). Incidence tables will be summarized with ATC Level 2 code, text and Drug Code, Drug Name.

The period of Concomitant medications use in each group is as follows.

Period 1: After administration in 1st Treatment to before administration in 2nd Treatment.

Period 2: After administration in 2nd Treatment to before administration in 3rd Treatment.

Period 3: After administration in 3rd Treatment to end of follow up.

7.5 Study Drug Exposure

All exposure data will be listed.

7.6 Treatment Compliance

All compliance data will be listed.

7.7 Statistical/Analytical issues

7.7.1 Adjustments of covariates

No statistical testing and estimation will be performed.

7.7.2 Handling of Dropouts or Missing Data

Missing data, such as rejected values, will not be imputed.

7.7.3 Interim Analyses and Data Monitoring

Not applicable in the study.

7.7.4 Multicentre Studies

Not applicable in the study.

7.7.5 Multiple Comparison/Multiplicity

No statistical testing and estimation will be performed.

7.7.6 Use of an "Efficacy Subset" of Patients

Not applicable in the study.

7.7.7 Active-Control Studies Intended to Show Equivalence

Not applicable in the study.

7.7.8 Examination of Subgroups

Not applicable in the study.

7.7.9 Handling of Routine safety laboratory tests Values

In the case of clinical laboratory test values including equality and inequality sign, exclude equality and inequality sign and use for summarized.

7.7.10 Handling of re-examination data

For the re-examination at screening, the examination value of the re-examination at the point closest to date of IMP is adopted.

If there is unscheduled data after the date of IMP, it will be excluded from calculation of summary statistics and frequency aggregation.

7.8 Pharmacokinetic Assessments

7.8.1 Analysis of Individual Plasma Concentrations

All measured plasma concentrations will be listed.

Plasma concentrations will be summarized at each scheduled sampling time point by each MCI-186 group (60 mg group and 300 mg group). The following descriptive statistics will be calculated: N (number of subjects), n (number of valid observations), arithmetic mean, SD, CV%, minimum, median, maximum, geometric mean and geometric CV%. Nominal sampling times will be displayed in the summary. For the calculation of the descriptive statistics other than geometric mean and geometric CV%, concentration values reported as BLQ will be set to 0. For the calculation of the geometric mean and geometric CV%, concentration values reported as BLQ will be set to ½ of LLOQ.

CV% and Geometric CV% will be calculated as follows:

$$\text{CV\%} = \frac{\text{SD}}{\text{arithmetic mean}} \times 100$$

$$\text{Geometric CV\%} = [\exp(\sigma^2) - 1]^{1/2} \times 100$$

where σ represents the SD computed on the natural logarithmic transformed concentrations.

To visualize the concentration-time profiles of each MCI-186 group, the following plots will be produced in linear and semi-logarithmic scales:

1. Individual subject observed and predicted concentration-time plot with each MCI-186 group (60 mg group and 300 mg group) overlaid in one graph by subject.
2. Individual subject observed concentration-time plot overlaid in one graph by each MCI-186 group (60 mg group and 300 mg group).
3. Mean observed and predicted concentration-time plot with each MCI-186 group (60 mg group and 300 mg group) overlaid in one graph.
4. Mean observed concentration-time plot with each MCI-186 group (60 mg group and 300 mg group) overlaid in one graph.

In the summary tables, arithmetic mean, SD, minimum, median, maximum and geometric mean will be presented with the number of significant digits which individual concentrations are reported. In addition, CV%, and geometric CV% will be presented with 1 decimal place.

7.8.2 Analysis of Pharmacokinetic Parameters

All PK parameters will be listed.

The PK parameters will be summarized by each MCI-186 group (60 mg group and 300 mg group). The following descriptive statistics will be calculated: N (number of subjects), n (number of valid observations), arithmetic mean, SD, CV%, minimum, median, maximum, geometric mean and geometric CV%.

For the descriptive statistics, the minimum and maximum will be presented according to following requirement:

- C_{max}, A, B, C will be presented with the number of significant digits they are reported with.
- Other PK parameters: will be presented with a fixed number of decimal places for each parameter. The number of decimal places is 2 decimal places corresponding to having 3 significant digits at the minimum by analyte.

Mean, SD, median and geometric mean will be presented with the number of decimals as follows.

- C_{max}, A, B, C: will be presented with 4 significant digits.
- Other PK parameters: will be presented with 2 decimal places.

CV% and geometric CV% will be presented with 1 decimal place.

7.9 Safety Assessments

No imputation will be made in case of missing values.

7.9.1 Adverse Events

Adverse events will be coded using MedDRA. All AEs for each subject, including multiple occurrences of the same event, will be presented in full in a comprehensive listing including start/stop times, verbatim term, Preferred Term, System Organ Class (SOC), dose level, severity, seriousness, relationship to treatment and outcome will be provided.

Duration of the AE and time to the AE occurrence from start of the IMP will be calculated and presented in days and time.
AE Occurrence from Start of IMP = Date of Onset - Date of Administration +1.
Duration of AE = Date of Resolution - Date of Onset +1.

AEs which start on or after dosing that are expressed or exacerbated are defined as treatment emergent adverse events (TEAEs).

In the tabulations, numbers of subjects with TEAEs will be counted by each treatment group (60 mg group, 300 mg group and placebo group).

The period of AE occurrence in each group is as follows.

Period 1: After administration in 1st Treatment to before administration in 2nd Treatment.

Period 2: After administration in 2nd Treatment to before administration in 3rd Treatment.

Period 3: After administration in 3rd Treatment to end of follow up.

Following summaries of TEAEs will be presented:

- Summary of TEAEs by SOC and PT
- Summary of TEAEs by SOC, PT and severity of event
- Summary of TEAEs by SOC, PT and maximum IMP relationship

Proportion of subjects with any TEAE, subjects with any adverse drug reactions, subjects with any TESAE (Treatment Emergent SAE), subjects with any serious adverse drug reactions, and subjects with any TEAE leading to discontinuation of the study will be summarized.

The summary will be sorted by International Agreed Order (IAO) for SOC and alphabetical order for PT (or by frequency from the highest to the lowest).

For summaries of AEs multiple occurrences of the same event within a subject will be counted once in the summaries by SOC and PT; multiple occurrences of the same event within a subject will be counted once in the maximum intensity category (severe > moderate > mild) and/or maximum drug relationship category (reasonable possibility>no reasonable possibility). If intensity or relationship is found to be missing the most severe occurrence will be imputed for that particular summary.

7.9.2 Routine safety laboratory tests

All routine safety laboratory tests parameter will be listed.

Clinical significance of routine safety laboratory tests findings will be evaluated by the Investigator with respect to pre-defined clinically relevant ranges taking into account the Investigator site's normal ranges. The routine safety laboratory tests data will be listed in full with clinically relevant values flagged (L=Lower than normal range, H=Higher than normal range or A=Abnormal if no reference range). A listing of routine safety laboratory tests values will be provided for subjects with any clinical significant findings (list relevant routine safety laboratory tests parameters only).

Lab parameter values and changes from baseline will be summarized descriptively by each treatment group (60 mg group, 300 mg group and placebo group) and analysis visit window. Overall evaluation (Clinical Assessment) for each routine safety laboratory tests will be summarized using frequency and percentage.

Shift tables will present the changes in clinically relevant categories from baseline to each scheduled post-baseline visit. The categories will be qualitative values for Urinalysis (glucose, protein, occult blood, ketone bodies, urobilinogen, erythrocytes, leukocytes, squamous epithelial cells).

7.9.3 Vital Signs and Body Weight

All vital sign and body weight data will be listed.

Vital signs (systolic blood pressure, diastolic blood pressure, pulse rate, body temperature) /body weight values and changes from baseline will be summarized descriptively by analysis visit window. Overall evaluation for vital sign will be summarized using frequency and percentage.

7.9.4 Routine safety 12-lead ECG

All Routine safety 12-lead ECG (heart rate, RR, PR, QRS, QT, QTcF, overall evaluation) parameters and findings will be listed.

Routine safety 12-lead ECG parameter values and changes from baseline will be summarized descriptively by analysis visit window. Overall evaluation for routine safety 12-lead ECG will be summarized using frequency and percentage.

For ECGs, number and percentage of subjects meeting the criteria listed below will be presented:

- QTcF > 500 msec at time point
- 500msec >= QTcF > 480 msec at time point
- 480msec >= QTcF > 450 msec at time point
- QTcF <= 450 msec at time point
- Change from baseline in QTcF > 30 msec
- Change from baseline in QTcF > 60 msec

7.9.5 Physical Examinations

All physical examinations data will be listed.

7.9.6 Withdrawals

All subjects who are withdrawn from the study will be listed.

7.9.7 Other Safety Assessments

Not applicable in the study.

8. CHANGES FROM THE PROTOCOL

We will not summarize smoking and alcohol use as baseline characteristics.

9. DATA NOT SUMMARISED OR PRESENTED

Not applicable in the study.

10. REFERENCES

1. C Garnett, K Needleman, J Liu, R Brundage and Y Wang. Operational Characteristics of Linear Concentration-QT Models for Assessing QTc Interval in the Thorough QT and Phase I Clinical Studies. Clinical Pharmacology & Therapeutics, 2016;100(2): 120-178.

11. VALIDATIONS

SAS® for Windows (release 9.2 or a later version) will be used for statistical analyses.

Phoenix® WinNonlin® (release 6.3 or a later version) will be used to calculate Pharmacokinetics Parameters.

The quality of statistical results will be ensured by double programming at [redacted]

12. LISTINGS, TABLES AND FIGURES

12.1 Listings

No.	Title of listing	Analysis Population/Dataset
16.2.1 – Subject Disposition		
	Randomization Details	All Subjects
	Subject Disposition	All Subjects
	Analysis Population	All Subjects
16.2.2 – Inclusion and Exclusion Criteria		
	Inclusion and Exclusion Criteria	All Subjects
16.2.3 – Demography and Baseline Characteristics		
	Demography	All Subjects
	Baseline Characteristics	All Subjects
16.2.4 – Medical History and medications		
	Medical history	All Subjects
	Prior and Concomitant Medications	All Subjects
16.2.5 – Exposure and Compliance		
	Study Drug Exposure and Compliance	All Subjects
16.2.6 – Pharmacokinetics		
	Blood Collection Time for Pharmacokinetic Evaluation	All Subjects
	Plasma Concentrations	All Subjects
	Plasma Pharmacokinetic Parameters - Non-Compartmental Analysis	All Subjects
	Plasma Pharmacokinetic Parameters - Compartmental Analysis	All Subjects
16.2.7 – Adverse Events		
	Adverse Events	All Subjects
16.2.8 – Routine safety laboratory tests Parameters		
	Routine Safety Laboratory tests - Hematology	All Subjects
	Routine Safety Laboratory tests - Biochemistry	All Subjects
	Routine Safety Laboratory tests - Coagulation	All Subjects
	Routine Safety Laboratory tests - Urinalysis	All Subjects
	Routine Safety Laboratory tests – Urinary Sediment	All Subjects
	Routine Safety Laboratory tests – Overall Evaluation	All Subjects
16.2.9 – Other Safety assessments		
	Physical Examinations, Body weight and BMI	All Subjects
	Vital Signs	All Subjects
	Routine Safety 12-Lead ECG	All Subjects

12.2 Tables

No.	Title of table	Analysis Population/Dataset
14.1 – Study		
	Subjects Disposition	All Subjects

No.	Title of table	Analysis Population/Dataset
	Analysis Population	All Subjects
	Demographics and Baseline Characteristics	Safety, PK
	Summary of Prior and Concomitant Medications	Safety
14.2 – Pharmacokinetics		
	Descriptive Statistics for Plasma Concentrations	PK
	Descriptive Statistics for Plasma Pharmacokinetic Parameters - Non-Compartmental Analysis	PK
	Descriptive Statistics for Plasma Pharmacokinetic Parameters - Compartmental Analysis	PK
14.3 – Safety		
	Summary of Treatment Emergent Adverse Events	Safety
	Treatment Emergent Adverse Events by System Organ Class and Preferred Term	Safety
	Treatment Emergent Adverse Events by System Organ Class, Preferred Term and Severity	Safety
	Treatment Emergent Adverse Events by System Organ Class, Preferred Term and Relation to Study Drug	Safety
	Summary of Routine safety laboratory tests - Hematology	Safety
	Summary of Routine safety laboratory tests - Biochemistry	Safety
	Summary of Routine safety laboratory tests - Coagulation	Safety
	Summary of Routine safety laboratory tests - Urinalysis	Safety
	Summary of Routine safety laboratory tests - Overall Evaluation	Safety
	Shift table of Routine safety laboratory tests - Urinalysis	Safety
	Body weight	Safety
	Vital Signs	Safety
	Vital Signs - Overall Evaluation	Safety
	Routine Safety 12-Lead ECG	Safety
	Routine Safety 12-Lead ECG - Overall Evaluation	Safety
	Routine Safety 12-Lead ECG - Number of Subject Meeting QTcF Criteria	Safety

12.3 Figures

No.	Title of figure	Analysis Population/Dataset
14.2 – Pharmacokinetics		
	Profile of Mean Plasma Concentrations – PK	PK
	Profile of Mean observed and predicted Plasma Concentrations - Compartmental Analysis - PK	PK
16.2.6 – Pharmacokinetics		
	Profile of Individual observed and predicted Plasma Concentrations by Subjects - Compartmental Analysis	PK

No.	Title of figure	Analysis Population/Dataset
	Profile of Individual observed Plasma Concentrations by Each Group	PK

13. REVISION HISTORY FOR SAP AMENDMENTS

Version 2.0 (25 January, 2019)

It reflected the results of the data review meeting on 17 January, 2019.

In List of PK Parameters, the PK parameters were matched with those described in PK Parameter Calculations in Appendix 1 and 2.
In Section 1, description of Section 3 has been deleted because Holter ECG analysis was not described.

At the beginning of Section 2, "In this SAP, the analyses with underline are not planed." was added, and the contents of analysis in Section 2.1.1, 2.1.3 and 2.1.4 were underlined.

APPENDIX 1 – CALCULATION OF PHARMACOKINETIC PARAMETER USING NON-COMPARTMENTAL ANALYSIS

- Actual blood sampling times for the assay of MCI-186 will be used in the calculation of pharmacokinetic parameters
- All concentrations below the LLOQ will be set at zero for pharmacokinetic calculations
- Calculation of pharmacokinetic parameters will be used 1/Y weighting of the data
- When λ_z is missing (or cannot be determined), $AUC_{0-\infty}$, $AUC\%_{ex}$, MRT, CL, V_{ss}, V_z, Lower limited of λ_z, Upper limited of λ_z and Number of λ_z points will not be calculated

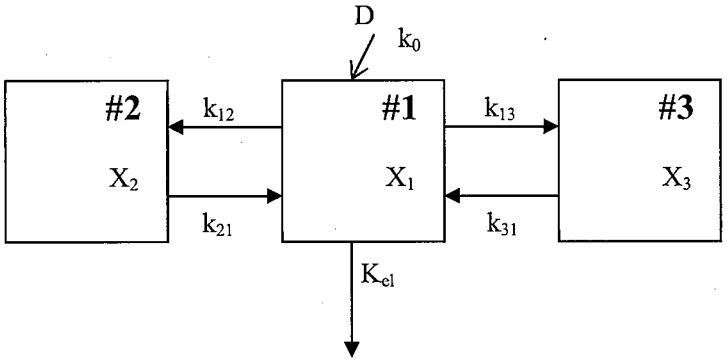
PK Parameter Calculations		
Parameters	Unit	Calculation
$AUC_{0\text{-last}}$	h•ng/mL	will be calculated using the linear trapezoidal method and actual times $AUC_{0-last} = \sum_{i=1}^{n} \frac{t_i - t_{i-1}}{2}(C_{i-1} + C_i)$
$AUC_{0\text{-}24h}$	h•ng/mL	will be calculated using the linear trapezoidal method and zero to 24h of actual times
$AUC_{0-\infty}$	h•ng/mL	$AUC_{0-\infty} = AUC_{0-last} + \frac{C_{last}}{\lambda_z}$
$AUC\%_{ex}$	%	$AUC\%_{ex} = \frac{AUC_{0-\infty} - AUC_{0-last}}{AUC_{0-\infty}} \times 100$
C_{max}	ng/mL	will be determined using maximum drug concentration
CL	L/h	$CL = \frac{Dose}{AUC_{0-\infty}}$

PK Parameter Calculations		
Parameters	Unit	Calculation
λ_z	1/h	The exponential rate constant of the terminal phase, λ_z, will be estimated by log-linear regression, if determinable. The number of data points included in the regression will be determined by visual inspection. Wherever possible, a minimum of 3 data points will be used in the estimation of λ_z. During the analysis, this calculation method repeats regressions using the last three points with non-zero concentrations, then the last four points, last five, etc. Points prior to C_{max} or prior to the end of infusion will not be used unless the user specifically requests that time range. Points with a value of zero for the dependent variable will be excluded. For each regression, an adjusted R^2 will be computed $$\text{Adjusted } R^2 = 1 - \frac{(1 - R^2) + (n - 1)}{(n - 2)}$$ where n is the number of data points in the regression and R^2 is the square of the correlation coefficient. The regression with the largest adjusted R^2 will be selected to estimate λ_z, with these caveats: If the adjusted R^2 does not improve, but is within 0.0001 of the largest adjusted R^2 value, the regression with the larger number of points will be used. λ_z must be positive, and calculated from at least three data points.
Lower limited of λ_z	h	will be determined using lower limit on time to be included in the calculation of λ_z
MRT	h	$$AUMC_{0-\infty} = \sum_{i=1}^{n} \frac{(t_i - t_{i-1})(t_i \times C_i + t_{i-1} \times C_{i-1})}{2} + \frac{t \times C_t}{\lambda_z} + \frac{C_t}{(\lambda_z)^2}$$ $$MRT = \frac{AUMC_{0-\infty}}{AUC_{0-\infty}} - \frac{TI}{2}$$ where TI represents infusion duration.
Number of λ_z points	-	will be determined using number of points used in computing λ_z. If λ_z cannot be estimated, zero.
$t_{1/2}$	h	$t_{1/2}$ will be determined as: $$t_{1/2} = \frac{\log_e(2)}{\lambda_z}$$

PK Parameter Calculations		
Parameters	Unit	Calculation
t_{max}	h	will be determined using time to maximum drug concentration
Upper limited of λ_z	h	will be determined using upper limit on time to be included in the calculation of λ_z
V_{ss}	L	$V_{ss} = MRT \times CL$
V_z	L	$V_z = CL \times \frac{1}{\lambda_z}$

APPENDIX 2 – CALCULATION OF PHARMACOKINETIC PARAMETER USING COMPARTMENTAL ANALYSIS

- Actual blood sampling times for the assay of MCI-186 will be used in the calculation of pharmacokinetic parameters.
- All concentrations below the LLOQ will be set at missing for pharmacokinetic calculations.
- In this model, use the three-compartment model as shown in the figure.



- Plasma concentration formula in infusion ($0 < t < T$) is as follows.

$$Cp(t) = \frac{A(1 - e^{-\alpha t})}{\alpha T} + \frac{B(1 - e^{-\beta t})}{\beta T} + \frac{C(1 - e^{-\gamma t})}{\gamma T}$$

- Plasma concentration formula after the end of infusion ($T \leq t$) is as follows.

$$Cp(t) = \frac{A(1 - e^{-\alpha T})}{\alpha T} e^{-\alpha(t-T)} + \frac{B(1 - e^{-\beta T})}{\beta T} e^{-\beta(t-T)} + \frac{C(1 - e^{-\gamma T})}{\gamma T} e^{-\gamma(t-T)}$$

A, B and C are coefficients which describe the exponential functions of each phase

α, β and γ are exponents which describe the shape of the curve for each phase

WinNonlin PK Parameter Calculation Settings	
Model Selection	Model 19 (Three-compartment, IV-infusion, and first-order elimination rate without lag time model)
Mappings	Subject id, Dose, Time, Concentration
Weighting	1/Yhat
Parameter Options	WinNonlin Generated Initial Parameter Values / WinNonlin Bounds
Minimization	Gauss-Newton (Levenberg and Hartley)
Increment for Partial Derivatives	0.001

WinNonlin PK Parameter Calculation Settings	
Meansquare	---
Iterations	50
Convergence criteria	0.0001

PK Parameter Calculations		
Parameters	Unit	Calculation
A	ng/mL	$A = \frac{D(k_{21} - \alpha)(k_{31} - \alpha)}{V_1(\gamma - \alpha)(\beta - \alpha)}$ An initial value is obtained from the above relational formula, and an estimated value is calculated using a nonlinear least squares method.
B	ng/mL	$B = \frac{D(k_{21} - \beta)(k_{31} - \beta)}{V_1(\gamma - \beta)(\alpha - \beta)}$ An initial value is obtained from the above relational formula, and an estimated value is calculated using a nonlinear least squares method.
C	ng/mL	$C = \frac{D(k_{21} - \gamma)(k_{31} - \gamma)}{V_1(\alpha - \gamma)(\beta - \gamma)}$ An initial value is obtained from the above relational formula, and an estimated value is calculated using a nonlinear least squares method.
α	1/h	$\alpha = \frac{k_{21} \cdot k_{31} \cdot k_{el}}{\beta \cdot \gamma}$ An initial value is obtained from the above relational formula, and an estimated value is calculated using a nonlinear least squares method.
β	1/h	$\beta = \frac{k_{21} \cdot k_{31} \cdot k_{el}}{\alpha \cdot \gamma}$ An initial value is obtained from the above relational formula, and an estimated value is calculated using a nonlinear least squares method.
γ	1/h	$\gamma = \frac{k_{21} \cdot k_{31} \cdot k_{el}}{\alpha \cdot \beta}$ An initial value is obtained from the above relational formula, and an estimated value is calculated using a nonlinear least squares method.

PK Parameter Calculations		
Parameters	Unit	Calculation
V_1	L	$V_1 = \frac{D}{A + B + C}$
k_{el}	1/h	$k_{el} = \frac{CL}{V_1}$
k_{21}	1/h	$k_{21} = \frac{\alpha \cdot \beta \cdot \gamma}{k_{el} \cdot k_{31}}$
k_{31}	1/h	$k_{31} = \frac{\alpha \cdot \beta \cdot \gamma}{k_{el} \cdot k_{21}}$
C_{max}	ng/mL	will be determined using maximum drug concentration
$t_{1/2\alpha}$	h	$t_{1/2\alpha} = \frac{\ln(2)}{\alpha}$
$t_{1/2\beta}$	h	$t_{1/2\beta} = \frac{\ln(2)}{\beta}$
$t_{1/2\gamma}$	h	$t_{1/2\gamma} = \frac{\ln(2)}{\gamma}$
$AUC_{0-\infty}$	h•ng/mL	$AUC_{0-\infty} = \frac{A}{\alpha} + \frac{B}{\beta} + \frac{C}{\gamma}$
CL	L/h	$CL = \frac{D}{AUC_{0-\infty}}$
MRT	h	$AUMC = \frac{A}{\alpha}\left(\frac{T}{2} + \frac{1}{\alpha}\right) + \frac{B}{\beta}\left(\frac{T}{2} + \frac{1}{\beta}\right) + \frac{C}{\gamma}\left(\frac{T}{2} + \frac{1}{\gamma}\right)$ $MRT = \frac{AUMC}{AUC_{0-\infty}}$
V_{SS}	L	$V_{SS} = CL \cdot MRT$