Protocol

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: 02.02
Date: August 29, 2018
NCT number: NCT04029090

STUDY PROTOCOL

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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

[redacted]

EudraCT Number:	Not applicable
IND Number:	126396
Investigational Medicinal Product:	MCI-186 (edaravone)
Development Phase:	Phase I
Sponsor:	Mitsubishi Tanabe Pharma Corporation (MTPC) 3-2-10, Dosho-machi, Chuo-ku, Osaka-shi, Osaka 541-8505, JAPAN
Protocol Version:	02.02
Protocol Date:	29 August 2018

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Cardiology Core Laboratory (pharmacodynamic [PD] ECG analysis)
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SIGNATURE PAGE (SPONSOR'S RESPONSIBLE SIGNATORY)

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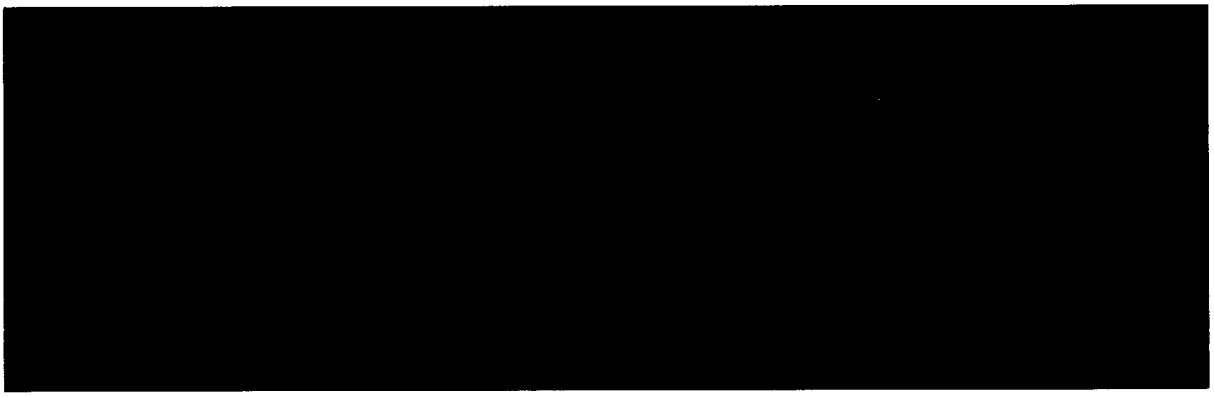
A Randomized, Single-Blind, Placebo-Controlled, Three-Way Crossover Study to Evaluate the Effect of MCI-186 at Therapeutic and Supra-Therapeutic Doses on the QT/QTc Interval in Healthy Subjects

The Protocol has been designed according to the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) Harmonised Tripartite Guideline for Good Clinical Practice (GCP), Japan GCP and the Declaration of Helsinki (Fortaleza, Brazil, 2013). It has undergone both medical and scientific review by competent Sponsor personnel.

Sponsor Signatory:



SIGNATURE PAGE (PRINCIPAL INVESTIGATOR)

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

I confirm that I have read this Protocol and understand its contents. I agree to fully comply with its requirements.

I agree to make no changes to the conduct of the study as defined by the Protocol without the prior authorization of Mitsubishi Tanabe Pharma Corporation in the form of a Protocol Modification and the appropriate regulatory and Institutional Review Board approvals.

Address of Institution: _____

_____

_____

_____

Signed: _____

Print Name: _____

Title: _____

Date: _____

LIST OF ABBREVIATIONS

Abbreviation	Definition
AE	Adverse event
AIS	Acute Ischemic Stroke
ALS	Amyotrophic lateral sclerosis
AUC	Area under the plasma concentration-time curve
BMI	Body mass index
CI	Confidence interval
C_{max}	Maximum plasma concentration
CS	Clinically significant
DCF	Data Clarification Form
ΔQTcF	Change from baseline in QTcF
ΔΔQTcF	Placebo-adjusted change from baseline in QTcF
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HIV	Human immunodeficiency virus
HR	Heart rate
IB	Investigator's Brochure
IC_{50}	Concentration associated with 50% inhibition
ICF	Informed Consent Form
ICH	International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use
IMP	Investigational Medicinal Product
IRB	Institutional Review Board
I.V.	Intravenous
Kg	Kilogram
kg/m^2	Kilogram per square meter
LS	Least squares
MedDRA	Medical Dictionary for Regulatory Activities
mg	Milligram
mL	Milliliter
μM	Micromolar concentraton
msec	Milliseconds
MTPC	Mitsubishi Tanabe Pharma Corporation
N	Number of subjects
n	Number of observations
NCS	Not clinically significant
NDA	New Drug Application
ng·hr/mL	Nanogram times hours per milliliter
ng/mL	Nanograms per milliliter
PD	Pharmacodynamic
PK	Pharmacokinetic(s)
PR	The PR interval of the ECG
QRS	The QRS interval of the ECG
QT	The QT interval of the ECG
QTc	Corrected QT interval
QTcB	Corrected QT interval using Bazett's formula
QTcF	Corrected QT interval using Fridericia's formula

Abbreviation	Definition
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SD	Standard deviation
SOC	System organ class
TEAE	Treatment-emergent adverse event
V2	Peripheral volume of distribution 2
w/v	Weight per volumne

PROTOCOL SYNOPSIS

Protocol number:	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.
Sponsor:	Mitsubishi Tanabe Pharma Corporation (MTPC) 3-2-10, Dosho-machi, Chuo-ku, Osaka-shi, Osaka 541-8505, JAPAN
Development phase:	Phase 1
Planned study period:	First subject first dose: October 2018 Last subject last visit: March 2019
Indication:	Not applicable
Investigational Medicinal Product:	MCI-186 supplied as 30 mg of edaravone per ampule (20 mL).
Reference product:	Placebo to match MCI-186: 0.9% w/v saline solution
Treatment regimen:	Treatment A (60 mg of MCI-186 as therapeutic dose) A single dose of 60 mg MCI-186 over 60 min will be intravenously administered. Treatment B (300 mg of MCI-186 as supra-therapeutic dose) 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.
Treatment duration:	Single dose for each treatment.
Objectives:	Primary Objective(s): • To evaluate the effect of MCI-186 on the QT interval corrected for heart rate using Fridericia's formula (QTcF) Secondary Objectives: • To evaluate the pharmacokinetic (PK) profile of MCI-186 • To evaluate the effect of MCI-186 on other 12-lead electrocardiogram (ECG) intervals • To evaluate the safety and tolerability of MCI-186
Study design:	A Randomized, Single-Blind, Placebo-Controlled, Three-Way Crossover Single-Center Study.
Planned number of subjects:	27 healthy male subjects
Subject population:	Healthy male subjects
Main inclusion criteria:	1. Healthy males aged 20 to 55 years (both inclusive) at signature of the Informed Consent Form (ICF). 2. Able to provide written informed consent to participate in this study after reading the ICF, and after having the opportunity to discuss the study with the Investigator or designee, before any screening or study related procedures take place. 3. In the Investigator's opinion, subject is able to understand the nature of the study and any risks involved in participation,

	and willing to cooperate and comply with the protocol restrictions and requirements. 4. A body weight of ≥45 kg and a body mass index (BMI) ranging from 18 to 30 kg/m^2 (both inclusive) at screening and Day -1. 5. Good health and free from clinically significant illness or disease in the opinion of the investigator on the basis of a physical examination, medical history, ECG, vital sign, and clinical laboratory test (biochemistry, hematology, coagulation and urinalysis) at screening and Day -1. 6. Male subjects must practice effective contraception during the study, from the time of the first dose of IMP until 14 days after the last dose of IMP.
Main exclusion criteria:	1. Subjects with PR >240 msec, QRS ≥120 msec, or QTcF >450 msec on the screening or Day -1 ECG, or any clinically significant electrocardiographic abnormality in the opinion of the Investigator. 2. Subject who has a history of cardiac disease or arrhythmias that can cause QTc prolongation. 3. Subject who has a family history of Torsade de Pointes, long-QT syndrome, hypokalemia or sudden death. 4. Subjects with potassium levels outside of the laboratory reference ranges at screening or Day -1. 5. Subjects with clinically significant deviations from normal in physical examination, vital signs, ECG or clinical laboratory test at screening or Day -1 in the opinion of the Investigator. 6. Presence or history of any clinically significant disease or organ dysfunction in the opinion of the Investigator. 7. Presence or history of allergy to food, any medical product or relevant excipient that is of clinical significant. 8. Subjects were previously administered MCI-186. 9. Presence or history of alcohol abuse or a positive alcohol test. 10. Presence or history of drug abuse or a positive drug screen test. 11. Positive test for hepatitis C virus antibody, hepatitis B surface antigen, human immunodeficiency virus (HIV) antigen/antibody or syphilis test at screening. 12. Participation in another trial within 12 weeks or 5 times the half-life of the drug whichever is longer before providing a signed ICF. For biologics, the minimum period is at least 24 weeks or the period of the pharmacodynamic effect, or 10 times the half-life of the drug, whichever is longer before providing a signed ICF. 13. Donate blood more than 200 mL within 4 weeks, 400 mL within 12 weeks or 1000 mL within 52 weeks, respectively before providing a signed ICF. 14. Donate plasma or platelet component within 2 weeks before providing a signed ICF. 15. Use of any prescription or non-prescription medications including herbal remedies and vitamin/mineral/protein

	supplements, except for acetylsalicylic acid, within 7 days prior to IMPs dosing. 16. Use of tobacco or nicotine containing products for 24 hours before each visit of screening or Day -1. 17. Consumption of alcohol, xanthines, or grapefruit containing products for 24 hours before each visit of screening or Day -1.
Triplicate ECG and PK Sampling	Continuous 12-lead Holter monitoring will be performed from at least 1 hour prior until 24 hours after dosing. From the Holter data, triplicate 12-lead pharmacodynamic (PD) ECGs of 10-seconds duration will be extracted at the following timepoints in relation to start of infusion: -45, -30 and -15 min; and 30, 60, 75, 90, and 105 min, and 2, 3, 4, 6, 8, 12 and 24 hours. PK samples for concentration of MCI-186 will be obtained predose, and after each of the ECG timepoints that occur after start of dosing.
Endpoints:	Primary Endpoint: • Relationship of change from baseline in QTcF (ΔQTcF) with placebo adjustment (ΔΔQTcF) and concentration of MCI-186 Secondary Endpoints: • Assessment of heart rate (HR), PR interval, QRS interval, and QTcF by timepoint • Categorical outliers for QTcF interval (absolute value and change from baseline) and other ECG parameters (if necessary) • 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
Statistical methods:	Sample size determination: The sample size for this study is not based on a formal statistical calculation. However, referring to the recent publication[6], 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. Pharmacodynamic ECGs: a) Concentration-response analysis: The primary outcome measure will be an analysis of the regression relationship between ΔQTcF and the plasma concentration of MCI-186 at matching times postdose,

	including adjustment for placebo, for the combined dose groups, as follows: • A linear mixed effects model with ΔQTcF as the dependent variable, MCI-186 plasma concentration as continuous covariate, the intercept, slope, influence of baseline on intercept, treatment (MCI-186=1 or placebo=0) and time from first administration as fixed effect parameters, intercept and slope as additive random effects. • It will be concluded that no significant repolarization effect was detected if the upper bound of the CI is <10 msec at the geometric mean C_{max} of both dose levels of MCI-186. • Point estimates for between-group difference in each MCI-186 group and combined placebo groups for ΔΔQTcF and corresponding 2-sided 90% confidence intervals will be constructed at the geometric mean maximum concentration (C_{max}) of each dose level of MCI-186, and for the quintiles of overall MCI-186 concentration. b) ECG interval analysis: Absolute value and change from baseline will be summarized by descriptive statistics (number of subjects (N), number of observations (n), mean, standard deviation (SD), median, minimum and maximum) for HR, PR interval, QRS interval, and QTcF intervals at each time point by each treatment. c) Categorical analysis: Categorical analysis will be performed to summarize the number and percentage of subjects meeting each criterion on each treatment. Absolute QTcF interval prolongation: ➢ QTcF interval >450 msec ➢ QTcF interval >480 msec ➢ QTcF interval >500 msec Change from baseline in QTcF interval: ➢ QTcF interval increases from baseline >30 msec ➢ QTcF interval increases from baseline >60 msec d) Morphological analysis: Morphological analysis will be performed to summarize the number and percentage of subjects in each treatment having the appearance of a morphological abnormality not present at baseline. PK: Plasma concentration-time profiles of MCI-186 for each subject and mean plasma concentration-time profiles will be plotted, plasma concentration data at each time point will be summarized by descriptive statistics, and PK parameters will be summarized with mean, medium, geometric mean, minimum, maximum, SD, and coefficient of variation.

	Safety: Where appropriate, continuous variables will be summarized descriptively, using N, n, mean, SD, median, minimum, and maximum. Categorical variables will be summarized using frequency counts and percentages. Treatment emergent adverse events will be coded using the latest available version of the Medical Dictionary for Regulatory Activities (MedDRA) and will be summarized in incidence tables by System Organ Class (SOC) and Preferred Term. Concomitant medication will be coded using the World Health Organization Drug Dictionary.

1 INTRODUCTION

MCI-186 (edaravone) is a non-peptide small molecule, synthetic compound with free radical scavenging effects. It exerts its neuroprotective activity by inhibiting the degradation of phospholipid membranes caused by free radicals. Mitsubishi Tanabe Pharma Corporation (MTPC) is developing MCI-186 for the treatment of amyotrophic lateral sclerosis (ALS) and acute ischemic stroke (AIS) in Japan. Further information can be found in the MCI-186 Investigator's Brochure (IB)[1].

MCI-186 was first approved in 2001 in Japan, under the trade name of RADICUT®, for the treatment of AIS using intravenous (I.V.) infusion of 30 mg MCI-186 administered over 30 minutes twice a day for up to 14 days of treatment. MCI-186 was designated as an orphan drug for treatment of ALS by the Japanese Minister of Health, Labour and Welfare in June 2005, the US Food and Drug Administration (FDA) in May 2015, and by the European Commission in June 2015.

MCI-186 was approved in Japan in June 2015 and in South Korea in December 2015 for the treatment of ALS based upon a series of clinical studies completed in Japan for ALS. The ALS dosing regimen is once a day I.V. infusion of 60 mg administered over 60 minutes following dosing cycles defined as follows: Cycle 1 consists of 14 consecutive treatment days followed by a 2 week drug-free period, all subsequent cycles consisting of 10 treatment days over 2 weeks followed by a 2-week drug-free period.

A New Drug Application (NDA 209176) for MCI-186 for the treatment of ALS was approved by FDA on 05 May 2017. In addition, FDA required MTPC to conduct a postmarketing clinical trial to assess the risk of QT prolongation with MCI-186 to exclude mean QTc effects greater than 20 msec.

1.1 Non-clinical pharmacology

Effects of MCI-186 on isolated cardiac tissues were investigated. A manual patch clamp study at body temperature in HEK293 transfected cells showed no effect (<5.0% inhibition) up to 100 μM of MCI-186 on the hERG-mediated cardiac potassium ion current[1]. From the results, at least a hundred-fold margin of safety compared to an anticipated therapeutic dose of 60 mg over 60 min is anticipated.

1.2 Clinical studies

I.V. infusion of MCI-186 has been evaluated in 21 studies (17 in Japan, 3 in Europe, and 1 in the Republic of Korea) comprising over 1700 subjects exposed to MCI-186, including approximately 100 healthy volunteers, 860 patients with AIS, 390 with subarachnoid hemorrhage, and 390 with ALS.

In I.V. administration of MCI-186, the maximum single dose tested was 1.5 mg/kg over 40 min achieving a maximum concentration (C_{max}) of 3061 ng/mL and the maximum multiple dose tested was 1 mg/kg over 40 min daily for 7 days achieving a range of C_{max} values 1616-1819 ng/mL[1]. These doses were well tolerated and no safety issues occurred or significant changes in laboratory results.

As of 8 August 2018, PK and safety of single orally administered MCI-186 (30 to 300 mg) have been investigated in healthy subjects in Japan. The preliminary results of C_{max} at the maximum single dose 300 mg achieved 8805 ng/mL. Eleven AEs occurred in edaravone group such as one event of nasopharyngitis in 30 mg of edaravone treatment, and one event of white blood cell increased and urinary occult blood positive in 120 mg of edaravone treatment, and one event of presyncope, conjunctivitis, creatine kinase increased, slight fever

and blood glucose level increased and two events of headache in 200 mg of edaravone treatment, and one event of white blood cell increased in 300 mg of edaravone treatment and 2 AEs in placebo group such as pharyngitis and uric acid increased. There were no safety concerns up to 300 mg in the preliminary results.

1.2.1 Cardiac Safety

In study MCI-186-E02 in healthy volunteers (N=33 active, N=13 placebo), an initial bolus of MCI-186 was followed by continuous infusions over 24 hours with total doses up to 12.0 mg/kg. The mean value of C_{max} in the highest dose group was 1165 ng/mL. This study had an adequate design to determine ECG findings, and the highest dose group had mean placebo-adjusted changes of QTc using the Bazett's formula (QTcB) from baseline between -12.5 and 2.7 msec during 48 hours of observation. Formal statistical comparisons between active and placebo findings showed no significant differences[3].

1.2.2 Population Pharmacokinetics

Study of population pharmacokinetics showed no significant differences between Japanese and non-Japanese populations except that race was statistically significant predictors of peripheral volume of distribution 2 (V2) from population PK (PPK) analysis[4]. The V2 of distribution was 26% higher for Caucasian subjects than for Japanese subjects. No significant differences were observed for C_{max} or AUC between the races from simulation of the PPK model. The simulated mean C_{max} following 14 daily doses 60 mg I.V. infused over 60 minutes was 1047 ng/mL.

1.2.3 Metabolism and Excretion

The major metabolites were the glucuronic acid conjugate and the sulfuric acid conjugate, and urinary excretion was the main metabolic pathway. The primary route of elimination is through the urine, with mean total urinary excretion of MCI-186 sulfate and MCI-186 glucronide (% of dose) of, respectively, 5.6–13.2 and 68.6–83.2[1].

With respect to inhibition of various P-450 molecular species present in human liver microsomes by MCI-186, the strongest inhibition occurred on CYP2C8/9. The sulfuric acid conjugate of MCI-186 is presumed to be subjected to deconjugation and then to glucuronidation in the human kidney and excreted in the urine. The inhibition rates of in vitro (human liver and kidney microsomes and liver S9) conjugation and metabolism of MCI-186 by coexisting drugs (furosemide, salicylic acid, metoclopramide, and acetaminophen) were not higher than 50%[1].

2 STUDY OBJECTIVES AND ENDPOINTS

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

3 STUDY DESIGN

3.1 Overall 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 as matching of 300 mg MCI-186 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

At baseline, at times of peak concentration and until 24 hours after the start of dosing, continuous 12-lead Holter monitoring will provide data for extraction of triplicate pharmacodynamic (PD) ECGs, and blood for determination of concentration of MCI-186 will be obtained.

All subjects will be admitted to the study center 1 day prior to dosing (Day -1) at each period. On Day 1, each subject will receive a single I.V. dose of either MCI-186 or placebo administered as a 1-hour infusion. Subjects will remain in the study center until the morning of Day 2. Next IMP administration will be separated with at least 72 hr and up to 9 days washout period after starting the previous administration. Subjects will return to the study center on Day 7 (+/- 2 days) of Period 3 as the Follow-up assessment.

3.2 Rationale for study primary endpoint

The update to ICH E14 of December 2015 supported the use of exposure-response analysis as the primary endpoint for such a trial. This analysis consists of regression modelling of placebo-adjusted changes of QTc from baseline as a function of concentration for all dose levels combined[5]. A supra-therapeutic dose is required to be included with the concentration level to be achieved based on the highest concentration anticipated during normal clinical use in the face of adverse alterations in metabolism or elimination at the therapeutic dose, termed the "high concentration scenario".

3.2.1 Rationale for endpoint criterion

FDA has required the Sponsor to assess the risk of QT prolongation with edaravone to exclude mean QTc effects greater than 20 msec. ICH E14[5] has required to be less than 10 msec as the upper bound of 90% confidence interval (CI) for the QTc effect at the highest clinically relevant exposure when using a exposure-response analysis. Therefore, the upper bound 90% CI is set to 10 msec according to FDA Advice Information Request letter (dated 19 Dec 2017).

3.3 Rationale for omitting a positive control group

No positive control is planned in accordance with ICH E14[5]. ICH E14 indicates a positive control would not be necessary if an appropriate supra-therapeutic dose can be set a sufficiently high multiple of the clinically relevant exposure.

3.4 Rationale for dose selection

The therapeutic dose of MCI-186 is 60 mg I.V. infused over 1 hour once daily for 14 days. Single doses are to be given in this study, with the therapeutic dose 60 mg I.V. infused over 1 hour, and the supra-therapeutic dose 300 mg I.V. infused over 1 hour.

Based on simulation data in healthy humans, a 60 mg infusion over 1 hour dose level of MCI-186 is anticipated to produce a maximum total plasma concentration of 1041 ng/mL. A dose of 300 mg over 1 hour is anticipated to produce a maximum total plasma concentration of 6117 ng/mL, see Figure 1. As previously noted, the maximum single dose tested by IV infusion, 1.5 mg/kg over 40 min gave C_{max} of 3061 ng/mL. The orally administered maximum single dose was 300 mg and this dose gave C_{max} of 8805 ng/mL. The both maximum doses were well tolerated and no safety issues occurred.

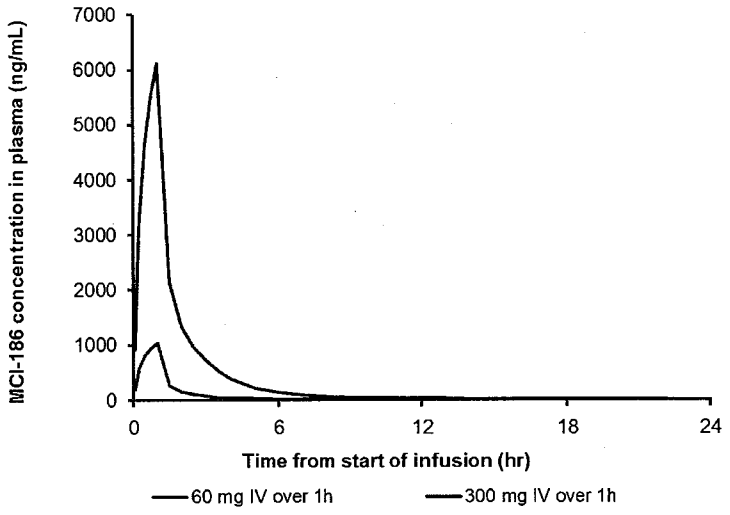


Figure 1: Simulated mean plasma concentration of MCI-186 after I.V. infusion

3.4.1 Rationale for single doses

Single doses were chosen as no accumulation is anticipated. Based on simulation data, the C_{max} after a single dose is anticipated to be 1047.3 ng/mL with an AUC of 1364.7 ng·hr/mL, and at steady state nearly identical values: 1049.0 ng/mL and 1373.9 ng·hr/mL.[4] While

multiple doses would potentially allow for metabolite accumulation, it was felt that the likelihood of the metabolites being cardioactive is small.

3.4.2 Other factors affecting high concentration scenario

There are no known effects affecting concentration for age or sex. Race had an effect on the volume of distribution but not on C_{max} or AUC. A human drug-drug interaction study was not conducted, but in vitro studies indicated that MCI-186 PK would be minimally affected by concomitant drugs. The sponsor is conducting two PK studies in subjects with mild and moderate renal or hepatic impairment in Japan (MCI-186-J22 or MCI-186-J23, respectively). The interim data indicates that renal or hepatic impairment may not increase in exposure to MCI-186. As a result of an exhaustive investigation about hepatic impairment effects for drugs which are conjugated by uridine diphosphate-glucuronyl transferase or sulfotransferase, which are involved in MCI-186 conjugation, the increase of exposure in most drugs (18/24) were within 2-fold. Only one drug (1/24) in the above investigation showed that the exposure in subjects with severe hepatic impairment were unexpected high (>5-fold) compared to those in healthy subjects. The sponsor assumes that the effect of severe hepatic impairment to MCI-186 exposure is also within 2-fold and sets 300 mg IV as the supra-therapeutic dose.

Thus, while the possible high clinical scenario concentration is uncertain, the supra-therapeutic dose chosen as 300 mg I.V. over 1 hour will give a C_{max} approximately 5 times the intended therapeutic dose.

3.5 Rationale for timing of observations

The maximum concentration is anticipated to be at the end of the infusion. The concentration will decrease sharply and will be nearly undetectable at 24 hours. The observations are focused at the time immediately after the end of infusion and extend out to 24 hours. Baseline PD ECG data will be collected in the hour prior to infusion at three separate timepoints. Triplicate ECG analysis will reduce the variability of the interval determinations. Concentration data will be obtained at baseline, at the mid-point of infusion, and immediately after each ECG timepoint.

3.6 Choice of healthy male volunteers

MCI-186 was well-tolerated at the doses chosen in healthy volunteers. Healthy volunteers will facilitate the collection of ECG data with less variability.

It is unlikely that unusual susceptibility to repolarization prolongation would be found in healthy females with clearly normal QTc values during screening and at baseline. Selection of only male volunteers is further justified as the effects of MCI-186 will be determined based on subject-specific baseline values.

3.7 Choice of QT correction

As no HR effects are anticipated, Fridericia correction will provide adequate adjustment for HR effects on the QT interval[5].

3.8 Cardiology Core Laboratory

A cardiology core laboratory highly experienced in such studies will provide centralized and standardized interpretation of PD ECGs. Advance systems will extract technically optimal ECGs from the continuous Holter data. ECG interpretation will be by manual adjudication

of semi-automated interval determination in a digital environment. A small number of expert MD electrocardiographers will be assigned, and a single reader will review all studies from any individual subject under blinded condition.

4 SELECTION AND WITHDRAWAL OF SUBJECTS

The Sponsor does not operate a Protocol waiver system for eligibility criteria.

4.1 Number of subjects

It is planned to randomize 27 subjects in this study.

4.2 Recruitment methods

Subjects will be recruited from a database of volunteers or *via* media advertisements, if appropriate. Subjects will be recruited according to study center's Standard Operating Procedures. All recruitment material will be approved by the Institutional Review Board (IRB) prior to implementation.

A sufficient number of subjects will be screened to ensure the planned sample size will be achieved. Each subject will be screened according to the criteria described in Sections 4.3 and 4.4. Only subjects who are eligible for the study will be randomized.

4.3 Inclusion criteria

A subject will be eligible for enrolment in the study if ALL of the following criteria apply:

1. Healthy males aged 20 to 55 years (both inclusive) at signature of the Informed Consent Form (ICF).
2. Able to provide written informed consent to participate in this study after reading the ICF, and after having the opportunity to discuss the study with the Investigator or designee, before any screening or study related procedures take place.
3. In the Investigator's opinion, subject is able to understand the nature of the study and any risks involved in participation, and willing to cooperate and comply with the protocol restrictions and requirements.
4. A body weight of ≥45 kg and a body mass index (BMI) ranging from 18 to 30 kg/m^2 (both inclusive) at screening and Day -1.
5. Good health and free from clinically significant illness or disease in the opinion of the investigator on the basis of a physical examination, medical history, ECG, vital sign, and clinical laboratory test (biochemistry, hematology, coagulation and urinalysis) at screening and Day -1.
6. Male subjects must practice effective contraception during the study, from the time of the first dose of IMP until 14 days after the last dose of IMP.

4.4 Exclusion criteria

A subject will NOT be eligible for this study if ANY of the following criteria apply:

1. Subjects with PR >240 msec, QRS ≥120 msec, or QTcF >450 msec on the screening or Day -1 ECG, or any clinically significant electrocardiographic abnormality in the opinion of the Investigator.
2. Subject who has a history of cardiac disease or arrhythmias that can cause QTc prolongation.
3. Subject who has a family history of Torsade de Pointes, long-QT syndrome, hypokalemia or sudden death.
4. Subjects with potassium levels outside of the laboratory reference ranges at screening or Day -1.

5. Subjects with clinically significant deviations from normal in physical examination, vital signs, ECG or clinical laboratory test at screening or Day -1 in the opinion of the Investigator.
6. Presence or history of any clinically significant disease or organ dysfunction in the opinion of the Investigator.
7. Presence or history of allergy to food, any medical product or relevant excipient that is of clinical significant.
8. Subjects were previously administered MCI-186.
9. Presence or history of alcohol abuse or a positive alcohol test.
10. Presence or history of drug abuse or a positive drug screen test.
11. Positive test for hepatitis C virus antibody, hepatitis B surface antigen, human immunodeficiency virus (HIV) antigen/antibody or syphilis test at screening.
12. Participation in another trial within 12 weeks or 5 times the half-life of the drug whichever is longer before providing a signed ICF. For biologics, the minimum period is at least 24 weeks or the period of the pharmacodynamic effect, or 10 times the half-life of the drug, whichever is longer before providing a signed ICF.
13. Donate blood more than 200 mL within 4 weeks, 400 mL within 12 weeks or 1000 mL within 52 weeks, respectively before providing a signed ICF.
14. Donate plasma or platelet component within 2 weeks before providing a signed ICF.
15. Use of any prescription or non-prescription medications including herbal remedies and vitamin/mineral/protein supplements, except for acetylsalicylic acid, within 7 days prior to IMPs dosing.
16. Use of tobacco or nicotine containing products for 24 hours before each visit of screening or Day -1.
17. Consumption of alcohol, xanthines, or grapefruit containing products for 24 hours before each visit of screening or Day -1.

4.5 Withdrawal of individual subjects

A subject will be withdrawn if ANY of the following criteria are met:

- The subject wishes to withdraw from further participation.
- The subject is significantly noncompliant with the Protocol.
- Continuing in the study would be detrimental to the subject's safety in the opinion of the Investigator, e.g.,
  - The subject experiences intolerable AEs or serious AEs (SAEs).
  - The subject has clinically significant changes in safety parameters at any of the post-dose time points, as confirmed with a repeat assessment performed as soon as possible after the initial out-of-range result.
  - The subject has an increase in QTcF to ≥500 msec or increase of ≥60 msec from baseline (pre-dose on Day 1), as confirmed with three consecutive ECGs taken at least five minutes apart in a 30-minute period.

In addition, a subject may be withdrawn at any time for reason(s) other than those listed here.

If a subject is discontinued prematurely from the study, the date the subject is withdrawn from the study and the reason for withdrawal will be recorded on the electronic case report form (eCRF).

In case of withdrawal of a subject, the Follow-up Visit assessments should be performed, as far as possible (Section 5.1.3).

Any withdrawal due to an AE or for any safety reason should be assessed for seriousness according to Section 8.2.

Any unresolved AE or SAE will be followed up according to Section 8.9.

In the event that a subject elects not to return to the study center for the Follow-up Visit, the Investigator must make every effort to contact the subject to review all AEs. In the event that a subject drops out of the study at any time, the reason for discontinuation must be fully documented in the source documents and the eCRF. The study center personnel will document the AEs and any other assessments in the source documents and will make every effort to complete all required Follow-up Visit assessments.

Subjects who are withdrawn from the study following Randomization may not re-enter the study.

Subjects withdrawn for non-treatment-related reasons may be replaced at the discretion of the Sponsor and Investigator. Replacement subjects will receive the treatments intended for the withdrawn subject.

4.6 Lifestyle restrictions

Subjects will be advised that they must adhere to the following restrictions by investigator and staff in the study center:

4.6.1 Attendance

- Subjects must be available to attend visits according to the Protocol.
- Subjects must be available for overnight stays in the study center for 2 nights in each 3 period.

4.6.2 Alcohol restrictions

- Subjects should refrain from consuming food or drink containing alcohol in the 24 hours before each visit and whilst confined to the study center.
- Subjects should avoid excessive consumption (>16 g as pure alcohol per day) of food or drink containing alcohol at all other times from the Screening visit until the Follow-up assessment.

4.6.3 Xanthines

- Subjects should refrain from consuming food or drink containing caffeine and methylxanthine, including coffee, tea, cola, energy drinks or chocolates in the 24 hours before each visit and whilst confined to the study center.
- Subjects should avoid excessive consumption (more than five cups of coffee or equivalent per day) of food or drink containing caffeine and methylxanthine (e.g., coffee, tea, cola, energy drinks or chocolates) at all other times from the Screening visit until the Follow-up assessment.

4.6.4 Other food restrictions

Subjects should refrain from consuming food or drink containing grapefruit (including marmalade and fruit juices) in the 24 hours before each visit and whilst confined to the study center.

4.6.5 Smoking

No smoking or using tobacco- or nicotine-containing products (snuff, chewing tobacco, cigarettes, cigars, pipes, e-cigarettes or nicotine replacement products) in the 24 hours before each visit and whilst confined to the study center.

4.6.6 Contraception

- Subjects (males) with partners of child-bearing potential must use a barrier method of contraception (i.e., male condom with spermicide) in addition to a second method of acceptable contraception used by their female partners. The additional contraception methods include:
    - Placement of an intrauterine device
    - Established use of oral hormonal methods of contraception associated with inhibition of ovulation
    - Male sterilisation (with the appropriate post-vasectomy documentation of the absence of sperm in the ejaculate).
    - Bilateral tubal ligation
    - Diaphragm with spermicide
- Subjects in a same sex relationship must use condom during sexual intercourse from the time of the first dose of IMP until 14 days after the last dose of IMP.
- Subjects must not donate sperm for the duration of the study, from the time of the first dose of IMP until 14 days after the last dose of IMP.
- Subjects must not have unprotected sexual intercourse during the study.

4.6.7 Diet

- While confined to the study center, subjects will receive standardised meals at scheduled times.
- Subjects will be required to fast (except for water) for at least 4 hours prior to routine safety blood sampling.
- No food or drink containing grapefruit (including marmalade and fruit juices) will be allowed in the 24 hours before each visit and whilst confined to the study center.
- Subjects should refrain from ingesting food or drink containing poppy seeds in the 72 hours before the Screening visit and Day -1 to avoid the occurrence of false positive opioid drug screen results.

4.6.8 Physical activity restrictions

- Must not participate in heavy physical training or excessive exercise (e.g., long distance running, weight lifting or any physical activity to which the subject is not accustomed) from seven days before first dose of IMP, during the study and until the Follow-up assessment.

4.6.9 Blood donation

- Donate blood more than 200 mL within 4 weeks, 400 mL within 12 weeks or 1000 mL within 52 weeks, respectively before providing a signed ICF.

- Donate plasma or platelet component within 2 weeks before providing a signed ICF.
- Subjects must agree not to donate blood for 3 months after the Follow-up assessment.

5 STUDY PLAN

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

Table 1 Time and events schedule

Study Period	**Screen-ing**	**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

5.1 Subject informed consent

Prior to performing any study procedures, the Investigator (or designated personnel) will ensure that the subject is given full and adequate oral and written information about the study and the subject must sign the ICF, as described in Section 11.2.1.

5.1.1 Screening

Screening assessments will be performed from Day -28 to Day -3.

At Screening, subjects will be requested to attend the study center after a 4-hour fasting period (apart from water). Written informed consent will be obtained before any screening procedures are performed. The following assessments will be performed (refer to Table 1 for further details):

- Written informed consent.
- Demography and medical history.
- Verify inclusion/exclusion criteria.
- Physical examination (including height, weight and BMI).
- Vital signs (including supine blood pressure, pulse rate and body temperature).
- Routine safety 12-lead ECG in triplicate.
- Screening for drugs of abuse and alcohol.
- Routine laboratory evaluations (hematology; biochemistry; coagulation; urinalysis).
- Serology.
- AE and prior and concomitant medication recording.

5.1.2 Confinement period

Subjects who successfully complete Screening will return to the study center on Day -1 will remain on-site until Day 2 in each 3 period. Inclusion and exclusion criteria will be reviewed to confirm eligibility on admission. No IMP will be administered on Day -1.

The following assessments will be performed (refer to Table 1 for further details and time points):

Day -1

- Verify inclusion/exclusion criteria.
- Abbreviated physical examination (including weight and BMI)
- Screening for drugs of abuse and alcohol.
- Vital signs (blood pressure, pulse rate and body temperature).
- Routine safety 12-lead ECG.
- Routine laboratory evaluations (hematology; biochemistry; coagulation; urinalysis).
- AE and concomitant medication recording.

Day 1 (pre-dose)

- Verify inclusion/exclusion criteria.
- Abbreviated physical examination

- Vital signs (blood pressure, pulse rate and body temperature)
- Routine safety 12-lead ECG.
- Eligible subjects will proceed to randomisation and dosing on Day 1 and remain in the study center until completion of assessments on Day 2.
- Rest period for 10 minutes prior to and for 5 minutes after each of the PD ECG timepoints at 45, 30 and 15 minutes before start of infusion.
- PK blood sampling for MCI-186.

Day 1 (post-dose)

- IMP administered over 1 hour
- Abbreviated physical examination
- Vital signs (blood pressure, pulse rate and body temperature) at 1 hour post-start of infusion.
- Routine safety 12-lead ECG at 1 hour post-start of infusion.
- Rest period for 10 minutes prior to and for 5 minutes after each of the PD ECG timepoints at 30, 60, 75, 90 and 105 minutes, and at 2, 3, 4, 6, 8 and 12 hours post-start of infusion.
- During each rest period, study personnel will check the integrity of Holter monitoring electrodes and functioning of the recorder.
- PK blood sampling for MCI-186 no sooner than 5 minutes but no later than 7 minutes after the timepoints in relation to start of infusion at 30, 60, 75, 90, and 105 min; and no sooner than 5 minutes but no later than 10 minutes after the timepoints in relation to start of infusion at 2, 3, 4, 6, 8 and 12 hours.
- AE and concomitant medication recording.

Day 2 (24 hours post-dose)

- Abbreviated physical examination (including weight).
- Vital signs (blood pressure, pulse rate and body temperature).
- Routine safety 12-lead ECG.
- Rest period for 10 minutes prior to and for 5 minutes after the PD ECG timepoint at 24 hours post-start of infusion.
- During the rest period, study personnel will check the integrity of Holter monitoring electrodes and functioning of the recorder.
- Discontinue Holter monitor no sooner than 5 minutes after the PD ECG timepoint at 24 hours post-start of infusion.
- Routine laboratory evaluations (hematology; biochemistry; coagulation; urinalysis).
- PK blood sampling for MCI-186 no sooner than 5 minutes and no later than 10 minutes after the ECG timepoint at 24 hours post-start of infusion.
- AE and concomitant medication recording.

5.1.3 Follow-up

Subjects will return to the study center between Day 5 -9 inclusive of Period 3 for a Follow-up Visit. The following assessments will be performed (refer to Table 1 for further details):

- Physical examination (including weight).
- Vital signs (blood pressure, pulse rate and body temperature).
- Routine safety 12-Lead ECG.
- Routine laboratory evaluations (hematology; biochemistry; coagulation; urinalysis).
- AE and concomitant medication recording.

5.1.4 Unscheduled visits

An unscheduled visit is defined as any visit to the study center outside of the Protocol-specified time points due to safety reasons or when a repeated measurement is required (e.g., obvious measurement errors, measuring device failure, confirmation of out-of-range results), where the subject is seen by study personnel.

Additional unscheduled samples for safety assessments may be performed at the discretion of the Investigator, if deemed necessary. All unscheduled visits and assessments performed during the visits will be recorded in the eCRF.

6 STUDY PROCEDURES

Procedures will be performed according to the time and events schedule (Table 1). A priority order will be in effect when more than one assessment is required at a particular time point, as follows: ECG extraction window (from nominal time to 5 minutes after nominal time), PK sample, vital signs, safety ECG. Time windows for relevant assessments will be described in a separate document.

6.1 Demography

Date of birth, sex, weight, height and race will be recorded in the eCRF.

6.2 Medical history

Any significant and relevant past conditions and any current medical conditions prior to Screening will be recorded in the eCRF.

6.3 Medication

At Screening, subjects will be asked what medications they have taken during the last 30 days. Only medication taken in the two weeks prior to administration of IMP will be recorded in the eCRF as prior medication.

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. All concomitant medications taken while the subject is participating in the study will be recorded in the eCRF with their daily dosage, route, duration, and reasons for administration.

6.3.1 Permitted medication

Medicines which, in the opinion of the Sponsor and Investigator, will not interfere with the study procedures or compromise safety may be used, e.g., acetylsalicylic acid (aspirin) for mild analgesia. However, any other concomitant medication may be given only if deemed strictly necessary for the subject's welfare by the Investigator.

6.3.2 Prohibited medication

Subjects must not participate in any other clinical study involving administration of an IMP for the duration of the current study.

Subjects must not take any prescribed or non-prescribed systemic or topical medication (including herbal remedies) unless, in the opinion of the Investigator and Sponsor, the medication will not interfere with the study procedures or compromise safety.

6.3.3 Rescue medication

There is no known antidote for MCI-186. Full resuscitation facilities will be available at all times.

6.4 Continuous 12-lead Holter electrocardiogram and Pharmacodynamic ECGs

Continuous 12-lead Holter data will be obtained from all subjects beginning at least 1 hour prior to and continuing at least until 24 hours after the start of infusion. Triplicate 10 second 12-lead PD ECGs will be extracted from the 5-minute period starting at the following timepoints in relation to start of infusion: -45, -30 and -15 min; and 30, 60, 75, 90, and 105 min, and 2, 3, 4, 6, 8, 12 and 24 hours.

Prior to each of these timepoints, subjects should observe at least 10 minutes of rest and rest will continue for 5 minutes after each timepoint. During each rest period, study personnel will check the integrity of Holter monitoring electrodes and functioning of the recorder. A detailed procedure will be described in a separate document.

Triplicate 12-lead PD ECGs of 10-second duration will be extracted from the Holter data by the cardiology core laboratory during the 5-minute period starting at each of the time points.

PK collection must be performed no sooner than 5 minutes after each of these times.

6.5 Pharmacokinetic assessments

Blood samples for MCI-186 will be collected pre-dose and no sooner than 5 minutes but no later than 7 minutes after the timepoints in relation to start of infusion at 30, 60, 75, 90, and 105 min; and no sooner than 5 minutes but no later than 10 minutes after the timepoints in relation to start of infusion at 2, 3, 4, 6, 8, 12 and 24 hours.

The analysis will be performed only on samples from subjects receiving active drug.

Blood samples will be packed in dry ice and sent by courier to:

[redacted]

Primary samples will be sent by courier from study center to [redacted] Contingency samples will be shipped after primary samples arrive.

6.5.1 Collection of blood samples for PK analysis of MCI-186

Blood samples will be collected via cannulation or direct venepuncture in a suitable forearm vein opposite to IMP administration at the times indicated in Table 1. The actual date and time of each blood sample will be recorded in the eCRF.

For each PK assessment, one blood sample of approximately four mL will be collected to ensure there is sufficient plasma for primary and contingency samples.

Sample handling details will be described fully in a separate document.

6.6 Safety assessments

Please refer to Section 8 for details of AE management. The results of all safety assessments will be recorded in the eCRF.

6.6.1 Physical examination

Physical examination, including body weight, height and BMI, will be assessed according to Table 1. The full physical examination will consist of a routine assessment of general appearance and major body systems: abdominal, cardiovascular, head, eyes, ears/nose/throat, lymph nodes, musculoskeletal, neck, neurological, dermatological, respiratory and 'other'.

The abbreviated physical examination will consist of a routine assessment of general appearance and of the following body systems: abdominal, cardiovascular, and respiratory.

Body weight will be measured with the subject wearing light clothing and without shoes. Height will be measured once only (at Screening). The BMI will be calculated using the standard formula at Screening and Day -1.

Body mass index calculation:

$$BMI = \text{Weight [kg]} / (\text{Height [m]})^2$$

6.6.2 Vital signs

Vital signs will be assessed according to Table 1. Subjects will undergo an assessment of supine blood pressure, supine pulse rate and infra-axillary body temperature.

Supine blood pressure will be measured using an automatic blood pressure recording device with an appropriate cuff size after the subject has rested for at least five minutes in a supine position. The same arm will be used for all measurements where possible.

The Investigator will perform an overall evaluation for safety purposes and the recording will be reported as 'normal', 'abnormal clinically significant (CS)' or 'abnormal not clinically significant' (NCS). Abnormalities of clinical significance will be reported as AEs. Repeat measurements will be performed, if needed.

6.6.3 Routine Safety Electrocardiogram

A routine safety 12-lead ECG will be performed using conventional bedside equipment according to Table 1 after the subject has rested for at least five minutes in a supine position.

The Investigator will perform an overall evaluation for safety purposes and the recording will be reported as 'normal', 'abnormal CS or 'abnormal NCS'. Clinically significant abnormalities will be reported as AEs. Repeat measurements will be performed, if needed.

6.6.4 Routine laboratory evaluations

Blood and urine samples will be collected for routine clinical laboratory safety evaluations according to Table 1. The laboratory safety evaluations performed during the study are presented in Table 2. Estimated GFR will be calculated using 3-variable Japanese equation in the study dataset.

Additional laboratory safety evaluations will be performed at other times, if judged to be clinically appropriate, or if the ongoing review of the data suggests a more detailed assessment of laboratory safety evaluations is required.

The Investigator will perform a clinical assessment of all laboratory safety data and the recording will be reported as 'normal', 'abnormal CS', or 'abnormal NCS'. Clinically significant abnormalities will be reported as AEs.

Table 2 Routine laboratory evaluations

Hematology:	
Hemoglobin	Mean corpuscular hemoglobin
Hematocrit	Mean corpuscular hemoglobin concentration
Platelet count	Mean corpuscular volume
Red blood cell count	White blood cell count – absolute and differential
Biochemistry:	
Alkaline phosphatase	Cholesterol
Aspartate aminotransferase	Triglycerides
Alanine aminotransferase	High density lipoprotein-cholesterol
Gamma-glutamyl transpeptidase	Low density lipoprotein-cholesterol
Potassium	Protein (total)
Sodium	Albumin
Chloride	Creatine kinase
Calcium	Creatinine
Inorganic phosphate	Lactate dehydrogenase
Glucose	Uric acid
Blood urea nitrogen	Amylase
Bilirubin (direct and total)	
Coagulation:	
Prothrombin time	Activated partial thromboplastin time
International normalised ratio	
Urinalysis:	
Specific gravity, sediment, pH, protein, glucose, ketones, urobilinogen, blood	
Microscopic examination[1]	
Serology:	
Hepatitis B surface antigen	HIV antigen/antibodies
Hepatitis C virus antibody	Syphilis test (RPR and TP antibody method)[2]
Drugs and alcohol screen:	
Urine phencyclidine, cocaine, barbiturates, tetrahydrocannabinol, benzodiazepines, amphetamines/methamphetamines, opiates and breath alcohol	

[1] Performed only if required, based on urinalysis results
[2] RPR: rapid plasma regain, TP: Treponema pallidum

Blood and urine samples will be analysed by the study center using standard methods. Laboratory safety assessments will be performed according to study center's Standard Operating Procedures.

6.7 Total blood volume

The approximate total blood volume taken per subject is given in Table 3.

Table 3 Blood volumes

Procedure	Sample volume (mL)	No. of samples	Total volume (mL)
Hematology	2	8	16
Biochemistry	8	8	64
Serology	11	1	11
Coagulation	1.8	8	14.4
Pharmacokinetics (MCI-186)	4	39	156
		Overall total	**261.4**

Additional or repeat safety and/or PK laboratory samples may be taken during the study if deemed necessary by the Investigator. The maximum volume to be drawn from each subject, including additional safety evaluations will be approximately 300 mL.

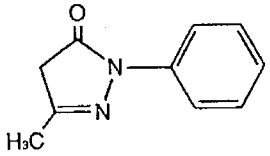
7 STUDY TREATMENT

7.1 Investigational Medicinal Product

A description of the IMP is given in Table 4.

Name and Structure of Drug Substance

Structural formula:



Nonproprietary name: Edaravone (JAN, r-INN)

Code name: MCI-186

Chemical name: 3-methyl-1-phenyl-2-pyrazolin-5-one

Molecular formula: $C_{10}H_{10}N_2O$

Molecular weight: 174.20

Pharmaceutical Properties and Formulation

The product is a clear and colorless aqueous injection containing 30 mg of edaravone in 1 ampule (20 mL). One ampule (20 mL) of the product contains as excipients 20 mg of sodium bisulfite, 10 mg of L-cysteine hydrochloride hydrate, 135 mg of sodium chloride, a suitable quantity of sodium hydroxide, and a suitable quantity of phosphoric acid.

Table 4 Investigational Medicinal Product and Placebo

	MCI-186	Placebo
Dosage form	Solution	Solution
Unit dose strength	30 mg edaravone per 20 mL ampule	0.9% w/v saline
Dosage Group	60 mg (2 ampules), 300 mg (10 ampules)	Placebo (total infusion volume to match of 60 mg and 300 mg MCI-186)
Route of administration	I.V.	I.V.
Dosing instructions	Infused over 1 hour	Infused over 1 hour
Storage conditions	Store at room temperature	Store at room temperature

IMP of MCI-186 will be manufactured by the Sponsor and provided as the commercially available drug product RADICUT® inj. (MCI-186 injection) 30 mg, compounded by Sawai Pharmaceutical Co., Ltd., Kashima Factory, Japan, and over-labelled.

Ten over-labelled ampules will be packed in a paper carton and certified by the Sponsor. IMP is tested and released according to Good Manufacturing Practice.

IMP of placebo will be provided as the commercially available 0.9% w/v saline by the Sponsor. Bags of placebo will be packed in a paper carton and certified by the Sponsor.

Both IMP of MCI-186 and placebo will be labelled on the paper carton and with the Sponsor's name and address, chemical name or identification code, manufacturing number and storage conditions. The label will contain the statement: "Investigational Product: to be used in a clinical investigation only" or other similar statement. The labelling will comply with applicable regulatory requirements.

7.1.1 Dosing

MCI-186 and placebo infusions should be administered in a fasted state by I.V. infusion over 1 hour. Investigator or designee will dilute 60 mg or 300 mg of MCI-186 and placebo with appropriate volume of physiological 0.9% saline. The total infusion volume in the three treatments will be the same volume. The actual started and completed time of administration will be recorded in the eCRF. A detailed procedure will be described in a separate document.

7.1.2 Compliance

IMP will be administered I.V. by Investigator or designated qualified study personnel at the study center who will check the subject's intravenous access prior to start of dosing and at regular intervals during dosing to confirm that the dose was properly infused. The Investigator, or suitably qualified staff member, will supervise the administration of IMP and the exact time of dosing will be recorded in the subject's source documents and eCRF.

The prescribed dosage, dose duration, timing and mode of administration of study medication may not be changed. Any departures from the intended regimen must be recorded in the eCRF.

7.1.3 Shipping, receipt, handling and storage

MCI-186 will be shipped from Mitsubishi Logistics Corporation to study center in a temperature-controlled shipping system after completion of a contract of the study between the Sponsor and study center.

On receiving the shipment of IMP at the study center, a qualified pharmacist or designee will conduct an inventory check and complete a supplies receipt document and a receipt of IMP returned to the Sponsor. A designee will maintain a record of all IMP received and returned.

At the study center, all IMP will be stored by the designee according to the conditions stated in the Procedure of IMP Management provided by the Sponsor in a locked, restricted access area. A temperature log recording a daily minimum/maximum temperature of the storage area will be maintained throughout the study. Any IMP storage temperature deviations will be reported to the Sponsor.

7.1.4 Dispensing

On each dosing occasion, the Investigator or designee will administer the allocated dose to the subject. A record of the IMP dispensed to each subject will be captured and maintained by the Investigator or designee. Any opened ampules will not be redispensed.

7.1.5 Accountability, returns and destruction

During the study, the designee will record the quantities of IMP dispensed and returned in an accountability log in accordance with the Procedure of IMP Management provided by Sponsor. IMP accountability (reconciliation) will be checked by the Sponsor. IMP is to be used only for this Protocol and not for any other purpose.

All unused IMP must be stored at the study center until it to be returned to the Sponsor.

7.2 Subject identification

Each subject will be assigned a unique Screening Identification Number by study center. The Investigator will keep a screening log of all subjects screened in order to assess the numbers and characteristics of the excluded subjects, and the reasons for their exclusion.

Upon randomization, each subject will also be assigned a unique Randomization Number. Both the Screening Number and the Randomization Number will be documented in the subject's source documents and eCRF. The Randomization Number will be used to identify subjects on IMP labels and other documentation.

Subjects who are withdrawn for non-treatment related reasons may be replaced at the discretion of the Sponsor and Investigator. The substitute subject will receive the same treatment assigned to the subject they replace. Subjects withdrawn as a result of treatment-emergent AEs thought to be causally related to the IMP will generally not be replaced.

A list identifying the subjects by the Screening Number and Randomization Number will be kept in the study center's file. The investigator will provide Screening list and Randomization list to the Sponsor when requested. In the provision, adequate attention should be paid to the privacy of patients and protection of personal information.

7.3 Procedures for assigning subjects to treatment groups

Randomization will be performed by the responsible person for allocation of IMP according to a computer-generated Randomization list, and subjects will be randomized in a 1:1:1 ratio to receive Sequence 1, Sequence 2 and Sequence 3 and also assigned a corresponding Randomization Number.

Randomization will take place after confirmation of inclusion and exclusion criteria prior to the first administration of IMP on Day 1.

The Randomization list and individual subject treatment assignments will be produced by the responsible person for allocation of IMP and provided to the Sponsor.

7.4 Maintenance of the study blind and unblinding

This study is a single-blind study. Subjects and ECG reviewer will be blinded. Investigator and Sponsor will be unblinded.

Treatment during all study parts will be single-blind, that is, the subject and ECG reviewer will not know which treatment is being infused (MCI-186 or placebo). The subject's I.V. delivery containers will be labelled with a unique number, which is traceable to the subject and treatments.

For each subject, randomization lists will be held in a secure area by the responsible person for allocation of IMP. If the blind is broken for any individual subject by the Investigator, the subject must be withdrawn from the study, and any procedures accompanying withdrawal should be performed (Section 4.5). The procedure for randomization and blinding in this study will be prepared by the responsible person for allocation of IMP and randomization of treatment sequence.

Due to PK analysis only being performed on samples from subjects receiving active IMP (MCI-186), the unblended Randomization list will be held by Bioanalytical Laboratory. (who will not have any influence on the clinical conduct of the study).

The Sponsor will authorise release of the unblinded Randomization list at the end of the study.

8 ADVERSE EVENT MANAGEMENT

All AEs and SAEs that occur from the time written informed consent is obtained until the end of the Follow-up assessment or the withdrawal of the subject from the study will be recorded in the source documents and eCRF. Even if the AE is assessed by the Investigator as not related to IMP, its occurrence must be recorded in the source documents and eCRF. AEs will be classified as 'baseline' if they occur before the administration of IMP. AEs will be classified as 'treatment-emergent' if they arise following the first administration of IMP or if a pre-dose AE increases in severity following dosing.

At each study visit, after the subject has had an opportunity to spontaneously mention any problems, the Investigator should inquire about the occurrence of AEs. The questioning should be open-ended and non-leading.

8.1 Definition of an adverse event

An AE is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product that does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of an IMP, whether or not considered related to the IMP.

8.2 Definition of a serious adverse event

An SAE is defined as any untoward medical occurrence that at any dose:

- Results in death.
- Is life-threatening.
- Requires hospitalisation or prolongation of existing hospitalisation.
- Results in persistent or significant disability or incapacity.
- Any event that is as serious as the above cases.
- Is a congenital anomaly or birth defect.

Medical and scientific judgement should be exercised in deciding whether an AE is serious and whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life threatening or result in death or hospitalization but may jeopardise the subject or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These should also be considered serious. Examples of such events are invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse. These should also usually be considered serious.

The term 'life threatening' refers to an event/reaction in which the subject was at risk of death at the time of the event/reaction; it does not refer to an event/reaction which hypothetically might have caused death if it were more severe.

Admission to a hospital as a new inpatient is deemed as meeting this criterion, even when the length of hospital stay was less than 24 hours. Transfer to other departments of the same hospital due to a newly emerged event during the hospitalization (e.g., transfer from the psychiatry ward to the internal medicine ward, from the internal medicine ward to the coronary intensive care unit, or from the neurology ward to the tuberculosis ward) is also counted as hospitalisation.

SAEs will be recorded and reported as described in Section 8.7.

8.3 Severity of adverse events

The severity of AEs will be classified according to the following criteria:

Mild: The event is transient and easily tolerated by the subject.

Moderate: The event causes discomfort and interferes with the subject's general condition.

Severe: The event causes considerable interference with the subject's general condition and may be incapacitating.

To ensure no confusion or misunderstanding of the difference between the terms 'serious' and 'severe', which are not synonymous, the following note of clarification is provided:

The term 'severe' is often used to describe the severity of a specific event (as in mild, moderate, or severe myocardial infarction); the event itself, however, may be of relatively minor medical significance (such as severe headache). This is not the same as 'serious', which is based on patient/event outcome or action criteria usually associated with events that pose a threat to a patient's life or functioning. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations.

8.4 Relationship of adverse events to Investigational Medicinal Product

The causal relationship of the AE to IMP will be determined as either 'reasonable possibility' or 'no reasonable possibility' defined as:

Reasonable Possibility – The relationship of the clinical event to the IMP makes a causal relationship possible, and other drugs, therapeutic interventions, or underlying conditions do not provide a sufficient explanation for the observed event.

No Reasonable Possibility – The relationship of the clinical event to the IMP makes a causal relationship unlikely, or other drugs, therapeutic interventions or underlying conditions provide a sufficient explanation for the observed event.

8.5 Clinical laboratory abnormalities and other abnormal assessments

Laboratory abnormalities that are CS will be recorded as AEs or SAEs. The Investigator will exercise medical judgment in deciding whether abnormal laboratory values are CS.

If an abnormal laboratory value or assessment is clearly related to a medically-defined diagnosis or syndrome, the diagnosis or syndrome will be recorded on the AE form, not the individual laboratory values.

All CS abnormal laboratory results or assessments will be followed until they resolve (return to normal or baseline values) or stabilise, or until they are judged by the Investigator to be no longer CS.

8.6 Recording and reporting of adverse events

All AEs, regardless of the relationship to IMP, occurring from the time written informed consent is obtained from a subject until the end of the safety Follow-up Period or the withdrawal of the subject from the study will be recorded.

All AEs will be recorded on an AE form in the eCRF. Reports should contain a description of the event, date and time of onset, date and time of resolution, severity, treatment required, relationship to IMP, action taken with the IMP, outcome and whether the event is classified as serious.

The Investigator will evaluate the severity of the AEs (as defined in Section 8.3) and will assess the causality between the AEs and the IMP (as defined in Section 8.4).

Pre-existing illnesses, which started prior to entry and are still ongoing at the start of the study, will not be considered AEs unless they worsen during the treatment period. Pre-existing conditions will be recorded as medical history.

If the Investigator becomes aware of any new safety information, or any safety information which appears to be either study or IMP related after the final Follow-up Period, then they must notify the Sponsor immediately.

8.7 Recording and reporting of serious adverse events

All SAEs occurring from the time written informed consent is obtained from a subject until the end of the safety Follow-up Period or the withdrawal of the subject from the study must be notified to the Sponsor, by email or fax, using a paper SAE form **within 24 hours** of the Investigator becoming aware of the SAE.

The SAE report should be completed as thoroughly as possible, including an assessment of causality. All such reports will identify subjects by unique code numbers assigned to the study participants, rather than by the subjects' names, personal identification numbers, or addresses. The SAE report should be signed and dated by the Investigator.

The reporting contact email address and fax number for SAEs will be described in a separate document.

Reports of pregnancy, although not classified as an SAE, will be handled and reported as described in Section 8.8.

The Sponsor will comply with the applicable regulatory requirements related to the reporting of suspected unexpected serious adverse reactions (SUSARs) to the regulatory authorities, the other Investigators and the Head of the other Institutions. The Investigator will be responsible for informing the Head of the study center of SAE or SUSARs, as per local laws and requirements.

8.8 Pregnancy

If a female partner of a male subject who has been exposed to the IMP becomes pregnant and the subject provides this information, then the pregnancy will be documented based on information provided by the subject.

Pregnancy, although not classified as an SAE, must be reported using the same timelines and contact details as an SAE (Section 8.7) but *via* a paper Mitsubishi Tanabe Pharma Pregnancy Notification Form. If the outcome or course of the pregnancy involves an SAE (e.g., a congenital anomaly), then an SAE form *via* fax needs to be completed in addition to the updated paper Mitsubishi Tanabe Pharma Pregnancy Notification Form. Termination of pregnancy for medical reasons, spontaneous abortion and congenital birth defects should always be reported as SAEs.

8.9 Follow up of adverse events

The Investigator should follow up subjects with AEs/SAEs, until the event has resolved or stabilised and any abnormal laboratory values have returned to baseline; or until there is a satisfactory explanation for the changes observed. If there are unresolved AEs at the Follow-up visit, the Investigator should follow up at least 21 days after Follow-up visit. In the case of death, if possible a pathologist's full report should be supplied.

8.10 Reference safety information

The reference source for safety information for this clinical study is the IB.

8.11 Overdose

Any subject who takes an overdose should be given the standard medical care (see Section 6.3.3).

If the subject takes a dose which is greater or more frequent than that specified in the Protocol (with or without associated symptoms), this must be reported to the Sponsor within 24 hours of awareness via a paper SAE form (using the contact details in Section 8.7).

If the subject experiences any associated symptoms as a result of the overdose, the Investigator will record this as a separate (S)AE.

9 DATA COLLECTION AND PROCESSING

9.1 Data collection

Subject data will be collected on individual eCRFs and will be substantiated by source documents (such as laboratory reports, medical records or safety ECGs) at the study center. Continuous 12-lead Holter ECG and PK data will be collected electronically and directly via [redacted] and [redacted]., respectively. All relevant data will be transcribed into the eCRF from source documents, entered into the study database directly from source documents or transferred electronically to the study database. Where no printed or electronic source documents exist, data will be entered directly into the eCRF and the eCRF will be considered the source document.

Prior to the start of the study, the Investigator will complete a Delegation of Responsibility List . The Sponsor will provide training for completion of the eCRF. The eCRF will be completed according to guidelines provided by the Sponsor or its designee in writing, electronically and/or verbally.

Completed eCRFs will be reviewed by the study monitor to ensure data accuracy, completeness and consistency. Any discrepancies found during the eCRF review or during data validation and/or quality assurance reviews of the data by data management or other functions are to be clarified by the Investigator (or his/her designated personnel).

The Investigator or designee must record all required subject data using the previously specified data collection method defined by the Sponsor. An explanation must be documented for any missing data. The Investigator must electronically sign and date a declaration on the eCRF attesting to his/her responsibility for the quality of all data recorded, and that the data represents a complete and accurate record of each subject's participation in the study. The data collected in the eCRF will be returned to the Sponsor, and an electronic copy will be retained by the Investigator.

9.2 Case Report Form completion

The eCRF will be presented in an electronic casebook comprising a series of electronic forms. The Subject Number should always be indicated and date (and time, if applicable) of each assessment should be entered in the eCRF.

The eCRFs must be completed in a timely manner so that this does not delay the ongoing data validation, review and quality control. The final, completed eCRF for each subject must be electronically signed and dated by the Investigator on the appropriate eCRF form to signify that he/she has reviewed the electronic casebook and certifies it to be complete and accurate.

9.3 Data processing

The data collected on the eCRFs will be captured in a specially constructed and validated database. The data will be validated using both manual and electronic means. Clarification of data will be requested from the study center as required. An audit trail of the original database entries, changes and deletions, session dates and times and the credentials of the database user who performed the operation will be maintained by the system. The completed database will be quality assured and locked to prevent further changes. A full database extract will be made available for statistical analysis according to the methods outlined in Section 10 and the Statistical Analysis Plan (SAP).

AEs and medical history entries will be coded using the Medical Dictionary for Regulatory Activities (MedDRA). Concomitant medications will be coded using the World Health

Organization Drug Dictionary. Versions of the dictionaries used will be documented in the Data Management Plan and SAP.

10 STATISTICAL METHODS AND PLANNED ANALYSES

10.1 Determination of sample size

Sample size determination:
The sample size of 27 subjects of IMP per treatments is not based on a formal power calculation. However, referring to the recent publication[6], 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.

10.2 Interim and Final Reporting

The study will have a final database lock. The final database lock will occur when all outstanding data queries have been resolved and the database is fully cleaned. This data will then be used to produce a full set of unblinded tables, figures and listings for the Clinical Study Report. The tables, figures and listings will then be communicated to the wider study team.

10.3 Study populations

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.
- PD population includes all randomized subjects who received at least one dose of IMP and who have at least one PD 12-lead ECG extracted post-dose.

The safety population will be used for all safety summaries. PK assessments will be performed on the PK population and PD assessments will be performed on the PD population.

10.4 Statistical analysis

10.4.1 General considerations

All individual subject data will be listed.

The statistical analysis will be performed using SAS® Version 9.2 or higher.

All variables will be summarized by treatment.

Unless otherwise stated, continuous data will be summarized descriptively including: number of subjects (N), number of observations (n), mean, standard deviation (SD), minimum, median and maximum. Categorical data will be summarized using frequency tables (frequency and percent).

The PK concentrations will be summarized by planned sampling time.

A detailed SAP will be prepared and approved prior to the study database lock which will include all detailed data handling and statistical methods. Any deviations from the planned analyses will be described and justified in the final integrated Clinical Study Report.

10.4.2 Missing Data handling

Procedures for the handling of any missing data will be described in the SAP.

10.4.3 Analysis of demography and other baseline subject characteristics

The number of subjects screened, randomized and included in each analysis population will be provided.

Demographic data and baseline characteristics such as age, ethnicity/race, height, body weight, BMI, smoking and alcohol use will be summarised. No formal statistical analysis of demographic or baseline characteristics will be performed.

Medical history will be coded using MedDRA latest version and listed for all subjects.

10.4.4 Pharmacodynamic ECGs

All interval values analysed will be the mean of the up to three replicate ECGs at each timepoint. Correction of QT for HR will be performed on each individual ECG prior to averaging using the Fridericia method:

$$QTcF = QT/RR^{(1/3)}$$ (with RR expressed in units of seconds).

10.4.5 Analysis of primary endpoint – PD ECGs Continuous 12-lead Holter extracted PD electrocardiogram

Data from continuous 12-lead Holter monitoring based on the extracted PD ECGs will be analyzed to investigate the potential effect of MCI-186 on the QTc interval.

Concentration-response analysis:
The primary outcome measure will be an analysis of the regression relationship between ΔQTcF and the plasma concentration of MCI-186 at matching times postdose, including adjustment for placebo subjects, for the combined dose groups, as follows:

- A linear mixed effects model with ΔQTcF as the dependent variable, the intercept, slope, infuence of baseline on intercept, treatment (MCI-186=1 or placebo=0) and time from first administration as fixed effect parameters, intercept and slope as additive random effects in below.

$$\Delta QTc_{i,j,k} = \theta_0 + \eta_{0,i} + \theta_1 TRT_j + (\theta_2 + \eta_{2,i})C_{i,j,k} + \theta_3 TIME_j + \theta_4(QTc_{i,j,k=0} - \overline{QTc_0})$$

Note:
$\Delta QTc_{i,j,k}$: the change from baseline in QTc for subject i in treatment j at time k
θ_0 : the population mean intercept in the absence of a treatment effect
$\eta_{0,i}$: the random effect associated with the intercept term θ_0
θ_1 : the fixed effect associated with treatment TRT_j (0=placebo, 1=MCI-186)
θ_2 : the population mean slope of the assumed linear association between concentration and $\Delta QTc_{i,j,k}$
θ_3 : the fixed effect associated with time
$\eta_{2,i}$: the random effect associated with the slope term θ_2
$C_{i,j,k}$: the concentration for subejct i in treatment j at time k
θ_4 : the fixed effect associated with baseline $QTc_{i,j,k=0}$
$\overline{QTc_0}$: the overall mean of all the baseline (=time 0) $QTc_{i,j,k=0}$values

It is assumed the random effects are bivariate normally distributed with mean [0,0] and an unstructured covariance matrix G, whereas the residuals are normally distributed with mean 0 and R

- Point estimates for between-group difference in each MCI-186 group and combined placebo groups (ΔΔQTcF) and corresponding 2-sided 90% CI will be constructed at the geometric mean C_{max} of each dose level of MCI-186, and for the quintiles of overall MCI-186 concentration.

It will be concluded that no significant repolarization effect was detected if the upper 90% CI of ΔΔQTcF are <10 msec at the geometric mean C_{max} for both dose levels of MCI-186.

10.4.6 Analysis of secondary PD ECG endpoints

10.4.6.1 ECG interval analysis:

Absolute value and change from baseline will be summarized by descriptive statistics (N, n, mean, SD, median, minimumand maximum) for HR, PR interval, QRS interval, and QTcF intervals at each time point by each treatment.

10.4.6.2 Categorical analysis:

Categorical analysis will be performed to summarize the number and percentage of subjects meeting each criterion on each treatment.

Absolute QTcF interval prolongation:

- QTcF interval >450 msec
- QTcF interval >480 msec
- QTcF interval >500 msec

Change from baseline in QTcF interval:

- QTcF interval increases from baseline >30 msec
- QTcF interval increases from baseline >60 msec

10.4.6.3 Morphological analysis:

Morphological analysis will be performed to summarize the number and percentage of subjects in each treatment having the appearance of a morphological abnormality not present at baseline.

10.4.7 Plasma concentration of unchanged MCI-186

Plasma concentration-time profiles of unchanged MCI-186 for each subject and mean plasma concentration-time profiles will be plotted, plasma concentration data at each time point will be summarized by descriptive statistics, and PK parameters will be summarized with mean, medium, geometric mean, minimum, maximum, standard deviation, and coefficient of variation.

10.4.8 Analysis of safety endpoints

All general safety variables will be summarized. All safety data will be listed. There will be no formal inferential statistical analysis of the general safety data.

Treatment emergent adverse events will be coded using the latest available version of the MedDRA and will be summarized in incidence tables by System Organ Class (SOC) and Preferred Term. Concomitant medication will be coded using the World Health Organisation Drug Dictionary.

10.4.8.1 Incidence of adverse events (AEs) and serious adverse events (SAEs)

Adverse events will be coded using MedDRA (version 20.0 or later). A by-subject AE data 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. All AEs that start before dosing will be classified as baseline AEs and will be listed only. All treatment-emergent AEs (TEAEs), i.e., AEs which start on or after dosing, will be tabulated. In the tabulations, numbers of subjects with TEAEs will be counted.

The following summaries of TEAEs will be presented:

- Summary of TEAEs by SOC and preferred term.
- Summary of TEAEs by SOC, preferred term and severity of event.
- Summary of TEAEs by SOC, preferred term and maximum IMP relationship.

In addition, details of AEs and SAEs leading to withdrawals will be listed.

10.4.8.2 Vital signs and routine safety electrocardiogram data

Vital signs and routine safety 12-lead ECG variables (without consideration of PD ECGs) and changes from baseline will be summarized at each time point by treatment.

The baseline for the vital sign parameters and routine safety 12-lead ECG measurements will be the last valid assessment obtained on or before Day 1 prior to the administration of single-blind IMP.

10.4.8.3 Routine safety laboratory tests

Routine laboratory safety tests include hematology, coagulation, biochemistry and urinalysis.

Continuous laboratory parameters will be summarized by scheduled time point and treatment groups. Observed values as well as changes from baseline (Day -1) will be summarized by treatment groups. Categorical parameters will be summarized by frequency counts and percentages of subjects within each category.

Clinical significance of abnormal laboratory findings will be evaluated by the Investigator with respect to pre-defined clinically relevant ranges taking into account the Investigator's normal ranges.

The laboratory 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 separate listing of laboratory values will be provided for subjects with any CS abnormal findings (only relevant laboratory parameters will be listed).

10.4.8.4 Physical examination

The data from the physical examination will be listed by subject. Changes in physical examinations will be described in the text of the clinical study report.

11 STUDY MANAGEMENT AND ETHICAL AND REGULATORY REQUIREMENTS

11.1 Good Clinical Practice

The Investigator will ensure that this study is conducted in compliance with the 2013 (Fortaleza, Brazil) revision of the 1964 Declaration of Helsinki. This study will also be conducted in accordance with Good Clinical Practice (GCP) requirements described in the current revision of International Conference on Harmonisation of Technical Requirements of Pharmaceuticals for Human Use (ICH) Guidelines and Japan-GCP. This study will also be carried out in accordance with regional and local legal requirements. Before the first subject is screened for the study, all ethical and legal requirements will be met.

11.2 Investigator responsibilities

11.2.1 Informed consent

The investigator will prepare an ICF. The ICF will be submitted to the Sponsor, and approved by the IRB prior to the start of the study.

Prior to undergoing any study-specific procedure, all legally competent subjects must consent in writing to participate. An ICF will be given to each subject, which will contain all regulatory-required elements, all ICH-required elements and data protection information, when applicable, in language that is understandable to the subject.

The process of obtaining the informed consent will be in compliance with all regulatory regulations, ICH requirements and local laws.

Either the Investigator or a designated person, qualified to meet any applicable local regulations, who is equally knowledgeable about the study will explain the aims, methods, anticipated benefits and potential hazards of the study and any discomfort it may entail. The review must be in a form understandable to the subject. A corresponding written explanation will also be provided and the subject allowed sufficient time to consider the study information.

If the subject is willing to participate in the study, the ICF will be sealed with printed name or signed and dated by the subject, the Investigator and, if applicable, the designated person who explained the nature of the study. The subject will receive a copy (together with the information sheet) and the original ICF will be retained with the study records at the study center.

The date (and time, if required) at which the ICF is signed by the subject must be recorded in the source notes and CRF.

The Investigator or his/her designee must emphasise to the subject that study participation is entirely voluntary and that consent regarding study participation may be withdrawn at any time without penalty or loss of benefits to which the subject is otherwise entitled.

If the ICF is amended during the study, the Investigator must follow all applicable regulatory requirements pertaining to approval of the amended ICF by the IRB. The study center personnel must use the amended ICF for all new subjects and repeat the consent process with the amended ICF for any ongoing subjects.

11.2.2 Contact to other attending physicians

The investigator will confirm during the Screening period whether subjects have medical attention by other doctors. When subjects are visiting his doctors, the investigator will inform his doctors of the participation in the study upon his consent.

11.2.3 Ethical and regulatory approval

The study must be conducted in accordance with ethical principles that have their origins in the Declaration of Helsinki and that are consistent with GCP as described in:

1. Declaration of Helsinki, concerning medical research in humans (Adopted by the 18th World Medical Association [WMA] General Assembly, Helsinki, Finland, June 1964 and amended by the: 29th WMA General Assembly, Tokyo, Japan, October 1975; 35th WMA General Assembly, Venice, Italy, October 1983; 41st WMA General Assembly, Hong Kong, September 1989; 48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996; 52nd WMA General Assembly, Edinburgh, Scotland, October 2000; 53rd WMA General Assembly, Washington DC, USA, October 2002 (Note of Clarification added); 55th WMA General Assembly, Tokyo, Japan, October 2004 (Note of Clarification added); 59th WMA General Assembly, Seoul, Republic of Korea, October 2008; 64th WMA General Assembly, Fortaleza, Brazil, October 2013).
2. ICH Harmonised Tripartite Guidelines for GCP 1996.
3. Japan-GCP
4. Law for Ensuring the Quality, Efficacy, and Safety of Drugs and Medical Devices.

The Investigator and Sponsor will sign this Protocol to confirm agreement to abide by it.

Before any study-related procedure is performed on a subject, all IRB, regulatory and local approvals of this Protocol and any other appropriate documents will be obtained. While the study is ongoing and at study completion/discontinuation, the Sponsor or Investigator will submit information to the IRB(s) in accordance with institutional/local regulations, for example:

- Information on SUSARs.
- Periodic reports on the progress of the study.
- Notification of the end of study or early termination.
- Final study summary upon completion or closure.

The Sponsor will ensure that any SUSARs from this study and other studies with this IMP are reported promptly to the regulatory authorities.

If it is necessary to amend the Protocol during the study, proper notification will be made to the regulatory authorities and IRB in the form of a Protocol Modification. Protocol Modification requiring IRB approval may be implemented only after a copy of the IRB's approval/favourable opinion letter has been transmitted to the Sponsor and regulatory authority approval has been obtained (if required). Protocol Modifications that are intended to eliminate an apparent immediate hazard to subjects may be implemented prior to receiving Sponsor, regulatory authority and/or IRB approval. However, in this case, approval must be obtained as soon as possible after implementation.

Any Protocol or other deviations that occur during the study will be documented and reported to the Sponsor. Depending on the nature of the deviation, this may be reported to the appropriate regulatory authority, the Head of the study center and IRB.

The Head of the study center will forward to the Sponsor and Investigator a copy of the written approval of the IRB and any other approving bodies.

11.2.4 Source document requirements and document access during the study

The Investigator or a designated person by the Head of the study center must retain a comprehensive and centralised filing system of all study-related documentation (including, but not limited to: essential documents, copies of Protocols, CRFs, source data such as original reports of test results, IMP dispensing logs, correspondence, records of informed consent and other documents pertaining to the conduct of the study) that is suitable for inspection by the Sponsor and representatives of regulatory authorities.

The Investigator/institution will permit study-related monitoring, audits, IRB reviews and regulatory inspections providing direct access to source data/documents.

11.2.5 Study records retention

Study-related documentation must be kept for at least 3 years or until notified by the Sponsor. Documents should be stored in such a way that they can be accessed/data retrieved at a later date. Consideration should be given to security and environmental risks.

No study document will be destroyed without prior written agreement between the Sponsor and the Investigator. Should the Investigator wish to assign the study records to another party or move them to another location, written agreement must be obtained from the Sponsor.

11.3 Study monitoring

In accordance with applicable regulations, GCP and the procedures of the Sponsor or its designees, the Study Monitor will periodically contact the study center, and conduct on-site visits. The extent, nature and frequency of on-site visits will be based on study complexity, enrolment rate and data quality at the study center. Through these visits and frequent communications (e.g., letter, email and telephone), the Study Monitor will verify that the investigation is conducted according to Protocol, regulatory and Sponsor requirements.

The Investigator and Head of study center will allow the Study Monitor direct access to all relevant documents, and allocate his/her time and the time of his/her personnel to the Study Monitor to discuss findings and any relevant issues.

In addition to contacts during the study, the Study Monitor will contact the study center personnel prior to the start of the study to discuss the Protocol and data collection procedures.

At study closure, the Study Monitor will conduct all activities as indicated in Section 11.5.

11.4 Quality assurance and auditing

Authorised representatives of the Sponsor, IRB and/or regulatory authorities may conduct an audit or inspection of this study either during or after completion. In such cases, the Investigator and Head of study center will give the auditor/inspector direct access to all relevant documents and source data, and will allocate his/her time and the time of his/her personnel as may be required to discuss findings and any relevant issues.

11.5 End of study and site closure

The end of the study is defined as the last visit for the last subject. Upon completion of the study, or if the study or study center is prematurely discontinued, the following activities, where applicable, must be conducted by the Study Monitor in conjunction with the Investigator and study center personnel:

- Return of all study data to the Sponsor.

- Completion of data clarifications and/or resolutions.
- Accounting, reconciliation and final disposition of used and unused IMP.
- Review of Investigator site study records for completeness.

11.6 Premature discontinuation of the study

The Sponsor reserves the right to discontinue the study because of safety concerns, ethical issues or serious and/or persistent non-compliance with the Protocol.

If the study is suspended or terminated by the Sponsor, the Sponsor will promptly inform the Head of study center and will also inform the regulatory authorities of the suspension or termination of the study and the reason(s) for the action. The Head of study center is responsible for promptly informing the Investigator and IRB, and providing the reason(s) for the suspension or termination of the study via a document.

If, in the opinion of the Investigator, the clinical observations in this study suggest that it may be unwise to continue, the Investigator may terminate part of, or the entire study.

If the study is suspended or terminated by the Investigator, the Investigator will promptly inform the Head of study center of the suspension or termination of the study and the reason(s) for the action via a document. The Head of study center is responsible for promptly informing the Sponsor and IRB, and providing the reason(s) for the suspension or termination of the study via a document.

If the study is suspended or terminated by the decision of the IRB, the IRB will promptly inform the Head of study center of the suspension or termination of the study and the reason(s) for the action via a document. The Head of study center is responsible for promptly informing the Investigator and Sponsor, and providing the reason(s) for the suspension or termination of the study via a document.

The Investigator is responsible for promptly informing the subjects and ensuring the subjects' safety.

For all subjects, the Follow-up Visit assessments should be performed, as far as possible (Section 5.1.3).

Any unresolved AE or SAE will be followed up according to Section 8.8.

In the event that a subject elects not to return to the study center for the end of study visit, the Investigator must make every effort to contact the subject to review all AEs. In the event that a subject drops out of the study at any time, the reason for discontinuation must be fully documented in the source documents and the CRF. The study center personnel will document the AEs and any other assessments in the source documents and the CRF, and will make every effort to complete all required end of study assessments.

In addition, all general study center activities required for the scheduled end of study and study center closure should be completed, as described in Section 11.5.

11.7 Premature discontinuation of study center

The Sponsor may at any time, at its sole discretion, discontinue the study center for various reasons, including, without limitation, the following:

- Failure of the Investigator to enrol subjects into the study at a reasonable rate.
- Failure of the Investigator to comply with applicable laws and/or pertinent regulations.

- Submission of knowingly false information from the research facility to the Sponsor, Study Monitor, or regulatory authorities.
- Insufficient adherence to Protocol requirements.

The Sponsor will issue a written notice to the Head of study center, which will contain the reasons for taking such action. If the study center is terminated for non-compliance, appropriate regulatory authorities will also be notified by the Sponsor.

For all subjects, the Follow-up Visit assessments should be performed, as far as possible (Section 5.1.3).

Any unresolved AE or SAE will be followed up according to Section 8.8.

In the event that a subject elects not to return to the study center for the end of study visit, the Investigator must make every effort to contact the subject to review all AEs. In the event that a subject drops out of the study at any time, the reason for discontinuation must be fully documented in the source documents and the CRF. The study center personnel will document the AEs and any other assessments in the source documents and the CRF, and will make every effort to complete all required end of study assessments.

In addition, all general study center activities required for the scheduled end of study and study center closure should be completed, as described in Section 11.5.

11.8 Liability and insurance

If study-related health damage occurs in subjects, except there is no causal relationship between the health damage and the study, the Sponsor will provide appropriate compensation based on the compensation standard by the Sponsor (contents of the compensation are defined as medical cost, medical allowance, and indemnity). In such cases, the Sponsor will not impose any burdens, such as proof of the causal relationship, to the subjects.

In order to ensure the implementation of compensation responsibility and liability for study-related injury to the subjects, the Sponsor will take appropriate actions including taking out insurance.

12 DISCLOSURE OF DATA

12.1 Confidentiality

A Subject Screening and Enrolment Log will be completed at study center for all subjects who signed an ICF. A Subject Identification Log, documenting the subjects' names, will be completed and retained at study center for all subjects enrolled in the study.

Subject names will remain confidential and will not be included in the database supplied to the Sponsor or its designee. If the subject name appears on any document collected, e.g., hospital discharge summary, the name must be obliterated before the document is transmitted to the Sponsor or its designee. All study findings will be stored in electronic databases. The subjects will give explicit permission for representatives of the Sponsor, regulatory authorities and the IRB to inspect their medical records to verify the information collected. Subjects will be informed that all personal information made available for inspection will be handled in the strictest confidence and in accordance with laws and regulations. All personnel involved in the study will observe and work within the confines of local data protection regulations.

All information concerning the product as well as any information such as clinical indications for the IMP, its formula, methods of manufacture and other scientific data relating to it, that have been provided by the Sponsor or designee, and are unpublished, are confidential and must remain the sole property of the Sponsor. The Investigator will agree to use the information only for the purposes of carrying out this study and for no other purpose unless prior written permission from the Sponsor is obtained. The Sponsor has full ownership of the data, including CRFs, obtained as part of the study.

12.2 Publication

By signing the study Protocol, the Investigator agrees that the results of the study may be used for the purposes of national and international registration, publication and information for medical and pharmaceutical professionals by the Sponsor. If necessary, the regulatory authorities will be notified of the Investigator's name, address, qualifications and extent of involvement.

The Sponsor or designee will prepare a final report on the study.

The Sponsor has the ownership of the information in the protocol, and the information will be provided to the study personnel, such as the Investigator or IRB, but the information must not be disclosed to any third party without agreement by the sponsor in writing, except when needed for the conduct of the study.

The study personnel at the study center such as the Investigator must obtain an approval from the Sponsor beforehand when they publish the results or information of this study.

The Sponsor can freely use the information obtained from this study for the purpose of reporting to regulatory authorities, and proper use and marketing of drugs.

13 REFERENCES

1. Edaravone Investigator's Brochure. Mitsubishi Tanabe Pharma Corporation, *Version 21, 29 June 2018.*
2. International Council for Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use - ICH HARMONISED TRIPARTITE GUIDELINE: The Clinical Evaluation of QT/QTc Interval Prolongation and Proarrhythmic Potential for Non-Antiarrhythmic Drugs – E14. *12 May 2005.*
3. Clinical Study Report: MCI-186-E02, *Final Version 3 April 2008*, p. 120.
4. Population Pharmacokinetic Analysis of MCI-186 in Japanese and Caucasians, Mitsubishi Tanabe Pharma Corporation *Final Version* 30 *October 2015.*
5. International Council for Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use - E14 Implementation Working Group ICH E14 Guideline: The Clinical Evaluation of QT/QTc Interval Prolongation and Proarrhythmic Potential for Non-Antiarrhythmic Drugs Questions & Answers *(R3) 10 December 2015.*
6. 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.