Statistical Analysis Plan

Protocol MT-1186-J02

Clinical Pharmacology Study of Oral Edaravone in Healthy Adult Males (Drug Interaction Study and Preliminary Regimen-Finding Study)

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POSITION:	COT STAT / COT CP
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Revision History

Version	Revision Comments
Ver1.0	First version
Ver1.1	The definition of PKPOP and SAF is changed in the section 5. With change of SAF definition, prior medications, concomitant medications in the section 7.7 and adverse events in the section 7.10.1 is changed.
Ver2.0	Final version

APPROVAL FORM

STATISTICAL ANALYSIS PLAN

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Date	[redacted]
Author	[redacted]

Mitsubishi Tanabe Pharma Corporation

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

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ABBREVIATIONS

AE	:	adverse event
ADR	:	adverse drug reaction
ALP	:	alkaline phosphatase
ALT	:	alanine transaminase
AST	:	aspartate transaminase
BLQ	:	Below level of quantification
BMI	:	body mass index
CI	:	confidence interval
CV	:	coefficient of variation
CRF	:	case report form
DP	:	decimal places
ECG	:	electrocardiogram
LLOQ	:	lower limit of quantification
LS	:	Least squares
MedDRA	:	medical dictionary for regulatory activities
PK	:	pharmacokinetics
SAP	:	statistical analysis plan
SD	:	standard deviation
SOC	:	system organ class
TFL	:	tables, figures and listings
ULN	:	upper limit of normal range
WHO	:	World Health Organisation

List of PK Parameters		
Parameters	**Unit**	**Definitions**
AUC_{0-t}	ng·h/mL	Area under the plasma concentration *vs* time curve from time zero up to the time of the last quantifiable concentration
AUC_{0-24}	ng·h/mL	Area under the plasma concentration *vs* time curve from time zero up to 24 hours
$AUC_{0-\infty}$	ng·h/mL	Area under the plasma concentration *vs* time curve from time zero extrapolated to infinity.
$AUC\%_{ex}$	%	Area under the plasma concentration-time curve extrapolated from the last quantifiable concentration time point to infinity in % of the total $AUC_{0-\infty}$
C_{max}	ng/mL	Maximum observed plasma concentration
CL/F	L	Apparent oral clearance
Kel	h^{-1}	Apparent terminal elimination rate constant
Lower limited of Kel	h	Lower data point used for the estimation of Kel
MRT	h	Mean residence time
Number of Kel points	–	Number of data point used for the estimation of Kel
$t_{1/2}$	h	Apparent terminal elimination half-life
t_{max}	h	Time to reach maximum plasma concentration
Upper limited of Kel	h	Upper data point used for the estimation of Kel
V_z/F	L	Apparent volume of distribution during the terminal phase after oral administration.
V_{ss}/F	L	Apparent volume of distribution at steady state after oral administration

1. INTRODUCTION

This statistical analysis plan (SAP) is based on the final protocol (v1.01) dated 15 October 2018. The plan covers statistical analysis, tabulations and listings of Pharmacokinetics(PK) and safety data.

The SAP is prepared by Mitsubishi Tanabe Pharma Corporation (MTPC). The statistical analyses and production of the outputs described in the SAP and QC will be conducted by [redacted] The final analyses and outputs will be approved by MTPC Data Science Department.

Any statistical analysis details described in this document supersede any description of statistical analysis in the protocol.

2. STUDY DESIGN

2.1 Phase and Type of the Study

Phase of the study: Period I

Type of study: Clinical pharmacology study

2.2 Study Design

2.2.1 Type and Details of Cohorts

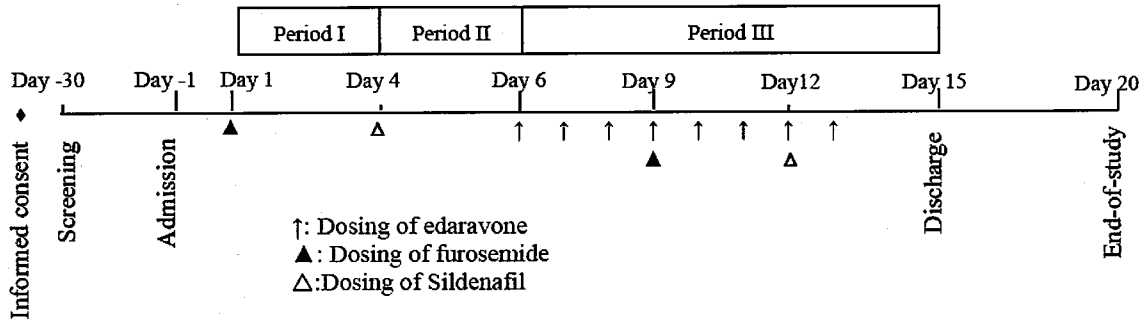
(1) Cohort 1: Drug interaction study

Open-label, add-on study (single dose of a victim drug, multiple doses of an perpetrator drug)

Group 1

	Period I (Dosing: Day 1)	Period II (Dosing: Day 4)	Period III (Dosing: Days 6 to 13)
Victim drugs	Rosuvastatin 10 mg, single dose	Sildenafil 50 mg, single dose	Rosuvastatin 10 mg, single dose (Day 9) Sildenafil 50 mg, single dose (Day 12)
Perpetrator drug	—	—	Edaravone 120mg*), multiple doses for 8 days (Days 6 to 13)

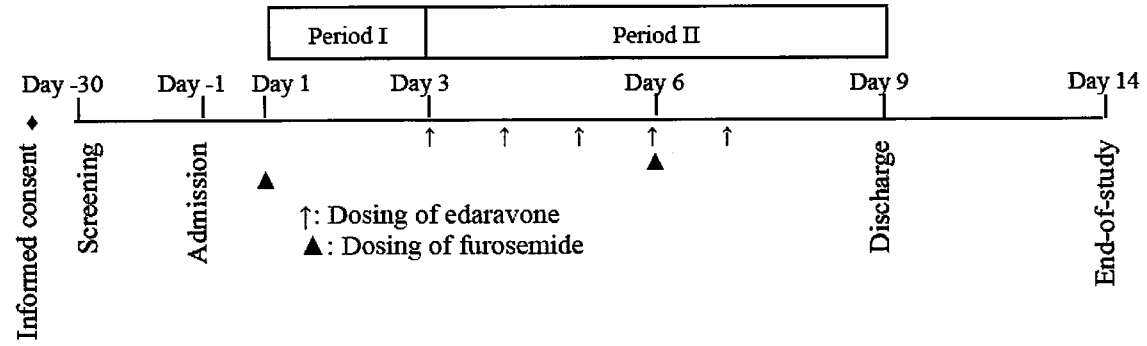
*) A provisional dose. Doses will be determined based on PK results of MCI-186-J25 study.



Group 2

	Period I (Dosing: Day 1)	Period II (Dosing: Days 3 to 7)
Victim drugs	Furosemide 40 mg, single dose	Furosemide 40 mg, single dose (Day 6)
Perpetrator drug	—	Edaravone 120 mg*), multiple doses for 5 days (Days 3 to 7)

*) A provisional dose. Doses will be determined based on PK results of MCI-186-J25 study.

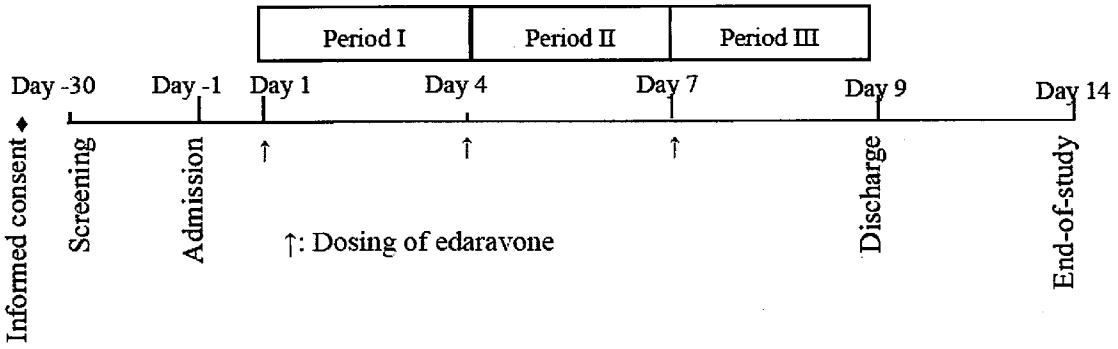


(2) Cohort 2: Preliminary regimen-finding study
Single-dose, open-label crossover study

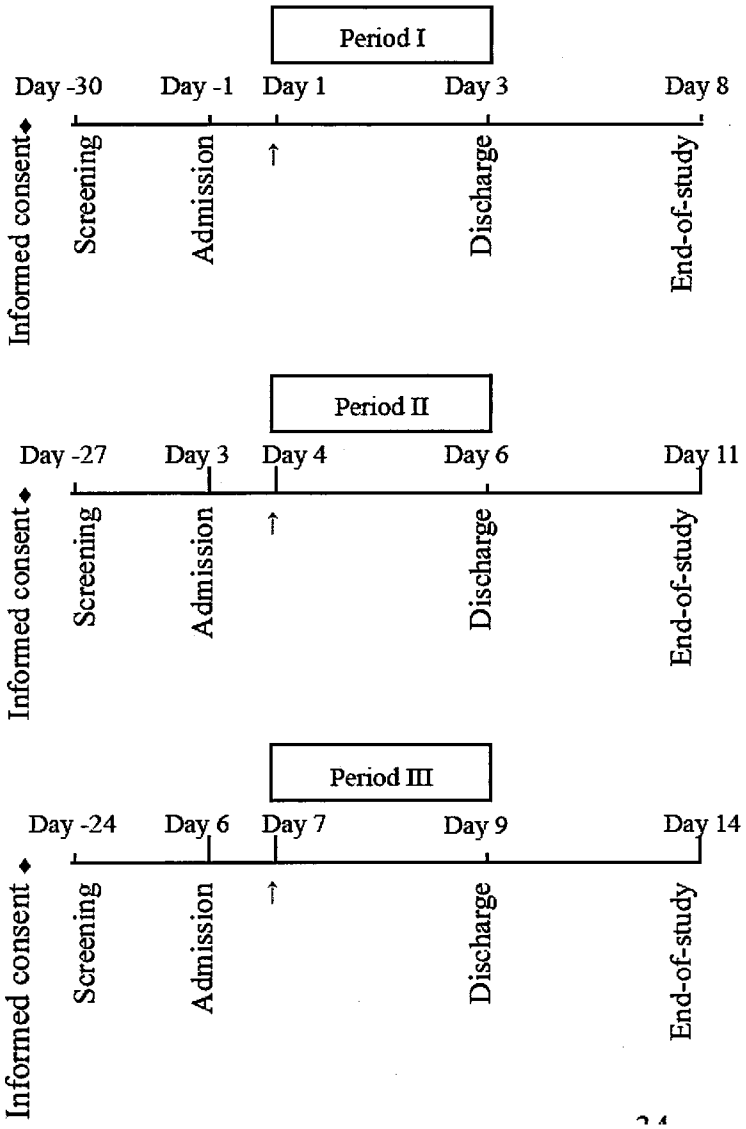
Group	Period I (Dosing: Day1)	Period II (Dosing: Day 4)	Period III (Dosing: Day 7)
3	Edaravone 90mg*), single dose No breakfast after dosing (3 Japanese and 3 Caucasians)	Edaravone 90mg*), single dose Start of a meal 1 hour after dosing (3 Japanese)	Edaravone 90mg*), single dose Dosing 4 hours after breakfast (3 Japanese)
4	Edaravone 90mg*), single dose Dosing 4 hours after breakfast (3 Japanese)	Edaravone 90mg*), single dose No breakfast after dosing (3 Japanese and 3 Caucasians)	Edaravone 90mg*), single dose Start of a meal 1 hour after dosing (3 Japanese)
5	Edaravone 90mg*), single dose Start of a meal 1 hour after dosing (3 Japanese)	Edaravone 90mg*), single dose Dosing 4 hours after breakfast (3 Japanese)	Edaravone 90mg*), single dose No breakfast after dosing (3 Japanese and 3 Caucasians)

*) A provisional dose. Doses will be determined based on PK results of MCI-186-J25 study.

Japanese subjects :



Caucasian subjects :



↑: Dosing of edaravone

(3) Study Period and Evaluation Period

Study period: The study period is defined as the period from informed consent to completion of the end-of-study assessment (or to completion or termination of follow-up, for any followed-up subjects).

Screening: Subjects providing informed consent will be screened for eligibility to select subjects meeting all of the inclusion criteria and none of the exclusion criteria (Cohort 1: 66 subjects [32 for Group 1 and 34 for Group 2], with a few reserve subjects; Cohort 2: 9 Japanese and 9 Caucasian subjects with a few reserve subjects).

Evaluation period: Cohort 1: The evaluation period is defined as the period from completion of dosing of the study drug or victim drugs (rosuvastatin, sildenafil, and furosemide) on Day 1 to completion of the end-of-study assessment. The duration of hospitalization will be 16 days and 15 nights (Day -1 to Day 15) for Group 1, and 10 days and 9 nights (Day -1 to Day 9) for Group 2. Cohort 2: The duration of hospitalization will be 10 days and 9 nights (Day -1 to Day 9) for Japanese subjects, and 4 days and 3 nights (Period I, Day -1 to Day 3; Period II, Day 3 to Day 6; Period III, Day 6 to Day 9) for Caucasian subjects.

End-of-study assessment: The prespecified observations and tests will be performed as the end-of-study assessment, 7 days (± 2 days) after the last dose of the study drug.

2.3 Test/Observation Schedule

(1) Cohort 1

1) Group 1

Day (time window)	Day of obtaining informed consent	Screening	Hospitalization																													
			Period I															Period II														
		Day -30 to -2	-1	1											2		3	4														5	
Time		Visit	Admission	8	8:30	9	10	11	12	13:30	14	16	19	20	0	8	8	8	8:15	8:30	9	9:30	10	11	12	13:30	14	16	19	20	8	20
Time (h) after dosing: edaravone																																
Time (h) after dosing: rosuvastatin				0	0.5	1	2	3	4	5.5	6	8	11	12	16	24	48															
Time (h) after dosing: sildenafil																		0	0.25	0.5	1	1.5	2	3	4	5.5	6	8	11	12	24	36
Screening		△																														
Written informed consent	X																															
Subject characteristics		△																														
Eligibility assessment		△	△	△																												
Dosing of edaravone																																
Dosing of rosuvastatin				X																												
Dosing of sildenafil																		X														
Meal on the days of dosing										X			X													X			X			
Height, weight, BMI[a)]		△	△																													
Physical examination		△	△	△		X			X					X		△	△	△			X				X					X	△	X
Vital signs		△	△	△		X			X					X		△	△	△			X				X					X	△	X
12-lead ECG		△	△	△		X			X					X		△	△	△			X				X					X	△	X
Laboratory tests		△	△														△														△	
Sensory tests																																
Adverse events				←																												
Concomitant medications																																
PK Blood sampling for edaravone																																
PK Blood sampling for rosuvastatin				△	X	X	X	X	X		X	X		X	X	X	X										X	X		X		
PK Blood sampling for sildenafil																		△	X	X	X	X	X	X	X		X	X		X	△	

△: To be performed before dosing of edaravone, rosuvastatin, or sildenafil, in the fasting state.

a) Height will be measured at screening only. Body weight will be measured at screening, hospitalization, and end-of-study assessments. BMI will be calculated at screening and hospitalization assessment.

Day (time window)	Hospitalization Period III																																				
	6													7						8						9												
Time	8	8:05	8:15	8:30	9	9:30	10	12	13:30	14	16	19	20	8	10	14	19	20	8	10	14	19	20	8	8:05	8:15	8:30	9	9:30	10	11	12	13:30	14	16	19	20
Time (h) after dosing: edaravone	0	0.083	0.25	0.5	1	1.5	2	4	5.5	6	8	11	12	0	2	6	11	12	0	2	6	11	12	0	0.083	0.25	0.5	1	1.5	2	3	4	5.5	6	8	11	12
Time (h) after dosing: rosuvastatin																								0	0.083	0.25	0.5	1	1.5	2	3	4	5.5	6	8	11	12
Time (h) after dosing: sildenafil	48																																				
Screening																																					
Written informed consent																																					
Subject characteristics																																					
Eligibility assessment																																					
Dosing of edaravone	X													X					X					X													
Dosing of rosuvastatin																								X													
Dosing of sildenafil																																					
Meal on the days of dosing									X			X			X	X	X			X	X	X											X			X		
Height, weight, BMI[a)]																																					
Physical examination	△				X			X					X	△				X	△				X	△				X				X					X
Vital signs	△				X			X					X	△				X	△				X	△				X				X					X
12-lead ECG	△				X			X					X	△				X	△				X	△				X				X					X
Laboratory tests																			△																		
Sensory tests	△																																				
Adverse events	←																																				
Concomitant medications																																					
PK Blood sampling for edaravone	△	X	X	X	X	X	X	X		X	X		X	△					△					△	X	X	X	X	X	X		X		X	X		X
PK Blood sampling for rosuvastatin																								△			X	X		X	X	X		X	X		X
PK Blood sampling for sildenafil	△																																				

△: To be performed before dosing of edaravone, rosuvastatin, or sildenafil, in the fasting state.

b) Height will be measured at screening only. Body weight will be measured at screening, hospitalization, and end-of-study assessments. BMI will be calculated at screening and hospitalization assessment.

Day (time window)	Hospitalization Period III																															End-of-study assessment[b]
	10						11					12																13					14	15	20 (±2)
Time	0	8	10	14	19	20	8	10	14	19	20	8	8:05	8:15	8:30	9	9:30	10	11	12	13:30	14	16	19	20	8	10	14	19	20	8	8	
Time (h) after dosing: edaravone	16	0	2	6	11	12	0	2	6	11	12	0	0.083	0.25	0.5	1	1.5	2	3	4	5.5	6	8	11	12	0	2	6	11	12	24	48	
Time (h) after dosing: rosuvastatin	16	24	26	30	35	36	48																										Visit
Time (h) after dosing: sildenafil												0	0.083	0.25	0.5	1	1.5	2	3	4	5.5	6	8	11	12	24	26	30	35	36	48		
Screening																																	
Written informed consent																																	
Subject characteristics																																	
Eligibility assessment																																	
Dosing of edaravone		X					X					X														X							
Dosing of rosuvastatin																																	
Dosing of sildenafil												X																					
Meal on the days of dosing			X	X	X			X	X	X											X			X			X	X	X				
Height, weight, BMI[a]																																	△
Physical examination		△				X	△				X	△				X				X					X	△				X	△	△	△
Vital signs		△				X	△				X	△				X				X					X	△				X	△	△	△
12-lead ECG		△				X	△				X	△				X				X					X	△				X	△	△	△
Laboratory tests							△																								△		△
Sensory tests																															△		
Adverse events	←																																→
Concomitant medications																																	→
PK Blood sampling for edaravone		△					△					△	X	X	X	X	X	X		X		X	X		X	△					△		
PK Blood sampling for rosuvastatin	X	△					△																										
PK Blood sampling for sildenafil												△		X	X	X	X	X	X	X		X	X		X	△					△		

△: To be performed before dosing of edaravone, rosuvastatin, or sildenafil, in the fasting state.

c) Height will be measured at screening only. Body weight will be measured at screening, hospitalization, and end-of-study assessments. BMI will be calculated at screening and hospitalization assessment.

d) At the time of withdrawal, assessment will be performed for the same items as those of the end-of-study assessment.

2) Group 2

Day (time window)		Informed consent Day of obtaining informed consent	Screening	Hospitalization														Hospitalization																					
				Period I														Period II																					
			Day -30 to -2	-1	1												2	3												4					5					
Time					8	8:30	9	9:30	10	11	12	13:30	14	16	19	20	8	20	8	8:05	8:15	8:30	9	9:30	10	12	13:30	14	16	19	20	8	10	14	19	20	8	10	14	19	20
Time (h) after dosing: edaravone			Visit	Admission															0	0.083	0.25	0.5	1	1.5	2	4	5.5	6	8	11	12	0	2	6	11	12	0	2	6	11	12
Time (h) after dosing: furosemide					0	0.5	1	1.5	2	3	4	5.5	6	8	11	12	24	36	48																					
Screening			Δ																																				
Written informed consent		X																																					
Subject characteristics			Δ																																				
Eligibility assessment			Δ	Δ	Δ																																		
Dosing of edaravone																			X												X				X				
Dosing of furosemide					X																																		
Meal on the days of dosing												X			X												X			X			X	X	X			X	X	X	
Height, weight, BMI[a)]			Δ	Δ																																			
Physical examination			Δ	Δ	Δ		X				X					X	Δ	X	Δ				X			X					X	Δ				X	Δ				X
Vital signs			Δ	Δ	Δ		X				X					X	Δ	X	Δ				X			X					X	Δ				X	Δ				X
12-lead ECG			Δ	Δ	Δ		X				X					X	Δ	X	Δ				X			X					X	Δ				X	Δ				X
Laboratory tests			Δ	Δ													Δ																				Δ				
Sensory tests																			Δ																						
Adverse events					←																																				
Concomitant medications																																									
PK	Blood sampling for edaravone																		Δ	X	X	X	X	X	X	X		X	X		X	Δ				Δ				
	Blood sampling for furosemide				Δ	X	X	X	X	X	X		X	X		X	X		Δ																						

Δ: To be performed before dosing of edaravone or furosemide, in the fasting state.

e) Height will be measured at screening only. Body weight will be measured at screening, hospitalization, and end-of-study assessments. BMI will be calculated at screening and hospitalization assessment.

Day (time window)		Hospitalization Period II																			End-of-study assessment[b]
		6													7					8	9	14 (±2)
Time		8	8:05	8:15	8:30	9	9:30	10	11	12	13:30	14	16	19	20	8	10	14	19	20	8	8	
Time (h) after dosing: edaravone		0	0.083	0.25	0.5	1	1.5	2	3	4	5.5	6	8	11	12	0	2	6	11	12	24	48	Visit
Time (h) after dosing: furosemide		0	0.083	0.25	0.5	1	1.5	2	3	4	5.5	6	8	11	12	24	2	6	11	36	48		
Screening																							
Written informed consent																							
Subject characteristics																							
Eligibility assessment																							
Dosing of edaravone		X													X								
Dosing of furosemide		X																					
Meal on the days of dosing											X			X			X	X	X				
Height, weight, BMI[a]																							Δ
Physical examination		Δ				X				X					X	Δ				X	Δ	Δ	Δ
Vital signs		Δ				X				X					X	Δ				X	Δ	Δ	Δ
12-lead ECG		Δ				X				X					X	Δ				X	Δ	Δ	Δ
Laboratory tests																					Δ		Δ
Sensory tests																					Δ		
Adverse events		←																					→
Concomitant medications		—																					→
PK	Blood sampling for edaravone	Δ	X	X	X	X	X	X		X		X	X		X	Δ					Δ		
	Blood sampling for furosemide	Δ			X	X	X	X	X	X		X	X		X	Δ					Δ		

Δ: To be performed before dosing of edaravone or furosemide, in the fasting state.

a) Height will be measured at screening only. Body weight will be measured at screening, hospitalization, and end-of-study assessments. BMI will be calculated at screening and hospitalization assessment.

b) At the time of withdrawal, the same tests will be performed as those of the end-of-study assessment.

(2) Cohort 2

1) Group 3: (Period I [No breakfast after dosing], Period II [Start of breakfast 1 hour after dosing], and Period III [Dosing 4 hours after the meal])

Day (time window)	Informed consent Day of obtaining informed consent	Screening	Hospitalization																	End-of-study assessment[d]
				Period I (No breakfast after dosing)														2	3	
		Day -30 to -2	-1	1																8 (±2)
Time		Visit	Admission	8	8:05	8:15	8:30	9	9:30	10	12	12:30	14	16	18	19	20	8	18	8	Visit[c]
Time (h) after dosing				0	0.083	0.25	0.5	1	1.5	2	4	4.5	6	8	10	11	12	24	36	48	
Screening		△																			
Written informed consent	X																				
Subject characteristics		△																			
Eligibility assessment		△	△	△																	
Dosing of edaravone				△																	
Meal on the days of dosing[a]												X				X					
Height, weight, BMI[b]		△	△																		△
Physical examination		△	△	△				X			X						X	△		△	△
Vital signs		△	△	△				X			X						X	△		△	△
12-lead ECG		△	△	△				X			X						X	△		△	△
Laboratory tests		△	△																	△	△
Adverse events				←	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	→
Concomitant medications				—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	→
PK Blood sampling for edaravone				△	X	X	X	X	X	X	X		X	X	X		X	△	X	△	

△: To be performed before dosing of edaravone or in the fasting state before breakfast.

○: Subjects will take a high-fat diet over 15 minutes.

b) Subjects will begin taking a meal after completing the prespecified blood sampling for PK and tests.

c) Height will be measured at screening only. Body weight will be measured at screening, hospitalization, and end-of-study assessments. BMI will be calculated at screening and hospitalization assessment.

d) The end-of-study assessment will be performed only in Caucasian subjects.

e) At the time of withdrawal, the same tests will be performed as those of the end-of-study assessment.

Day (time window)	Hospitalization																	Hospitalization																		End-of-study assessment[h]
	Period II (Start a meal 1 hour after dosing)																	Period III (Dosing 4 hours after completion of a meal)																		
	4														5		6	7																8		9		14 (±2)
Time	8	8:05	8:15	8:30	9	9:30	10	12	13:30	14	16	18	19	20	8	18	8	6:45	11	11:05	11:15	11:30	12	12:30	13	15	17	19	20	21	23	11	23	8:30	11	Visit
Time (h) after dosing	0	0.083	0.25	0.5	1	1.5	2	4	5.5	6	8	10	11	12	24	36	48	-4.25	0	0.083	0.25	0.5	1	1.5	2	4	6	8	9	10	12	24	36	45.5	48	
Screening																																				
Written informed consent																																			
Subject characteristics																																			
Eligibility assessment																																			
Dosing of edaravone	X																		X																
Meal on the days of dosing[a]					O				X				X					O								X			X						
Height, weight, BMI[b]																																			Δ
Physical examination	Δ				X			X						X	Δ		Δ		Δ				X			X				X	X			X	Δ
Vital signs	Δ				X			X						X	Δ		Δ		Δ				X			X				X	X			X	Δ
12-lead ECG	Δ				X			X						X	Δ		Δ		Δ				X			X				X	X			X	Δ
Laboratory tests																	Δ																Δ		Δ
Adverse events	←																																		→
Concomitant medications																																			>
PK Blood sampling for edaravone	Δ	X	X	X	X	X	X	X		X	X	X		X	Δ	X	Δ		Δ	X	X	X	X	X	X	X	X	X		X	X	X	X		X	

Δ: To be performed before dosing of edaravone or in the fasting state before breakfast.

O: Subjects will take a high-fat diet over 15 minutes.

f) Subjects will begin taking a meal after completing the prespecified blood sampling for PK and tests.

g) Height will be measured at screening only. Body weight will be measured at screening, hospitalization, and end-of-study assessments. BMI will be calculated at screening and hospitalization assessment.

h) The end-of-study assessment will be performed only in Caucasian subjects.

i) At the time of withdrawal, the same tests will be performed as those of the end-of-study assessment.

2) Group 4: (Period I [Dosing 4 hours after the meal], Period II [No breakfast after dosing], and Period III [Start of breakfast 1 hour after dosing])

Day (time window)	Informed consent Day of obtaining informed consent	Screening	Hospitalization																		
				Period I (Dosing 4 hours after completion of a meal)																	
		Day -30 to -2	-1	1														2		3	
Time		Visit	Admission	6:45	11	11:05	11:15	11:30	12	12:30	13	15	17	19	20	21	23	11	23	8:30	11
Time (h) after dosing				-4.25	0	0.083	0.25	0.5	1	1.5	2	4	6	8	9	10	12	24	36	45.5	48
Screening		Δ																			
Written informed consent	X																				
Subject characteristics		Δ																			
Eligibility assessment		Δ	Δ		Δ																
Dosing of edaravone					X																
Meal on the days of dosing[a)]				O								X			X						
Height, weight, BMI[b)]		Δ	Δ																		
Physical examination		Δ	Δ		Δ				X			X					X	X			X
Vital signs		Δ	Δ		Δ				X			X					X	X			X
12-lead ECG		Δ	Δ		Δ				X			X					X	X			X
Laboratory tests		Δ	Δ																	Δ	
Adverse events					←	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	→
Concomitant medications				—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	→
PK Blood sampling for edaravone					Δ	X	X	X	X	X	X	X	X	X		X	X	X	X		X

Δ: To be performed before dosing of edaravone or in the fasting state before breakfast.

O: Subjects will take a high-fat diet over 15 minutes.

j) Subjects will begin taking a meal after completing the prespecified blood sampling for PK and tests.

k) Height will be measured at screening only. Body weight will be measured at screening, hospitalization, and end-of-study assessments. BMI will be calculated at screening and hospitalization assessment.

l) The end-of-study assessment will be performed only in Caucasian subjects.

m) At the time of withdrawal, the same tests will be performed as those of the end-of-study assessment.

Day (time window)	Screening	Hospitalization																	End-of-study assessment[o]	Hospitalization																				End-of-study assessment[o]
			Period II (No breakfast after dosing)																		Period III (Start a meal 1 hour after dosing)																			
	Day -27 to 2	3	4														5	6	11 (±2)	7																8	9	14 (±2)
Time	Visit[o]	Admission[o]	8	8:05	8:15	8:30	9	9:30	10	12	12:30	14	16	18	19	20	8	18	8	Visit[o]	8	8:05	8:15	8:30	9	9:30	10	12	13:30	14	16	18	19	20	8	18	8	Visit
Time (h) after dosing			0	0.083	0.25	0.5	1	1.5	2	4	4.5	6	8	10	11	12	24	36	48		0	0.083	0.25	0.5	1	1.5	2	4	5.5	6	8	10	11	12	24	36	48	
Screening	△																																					
Written informed consent																																						
Subject characteristics	△																																					
Eligibility assessment	△	△	△[o]																																			
Dosing of edaravone			X																	X																	
Meal on the days of dosing[n]											X				X														X				X				
Height, weight, BMI[b]	△	△																	△																		△
Physical examination	△	△	△				X			X						X	△		△	△	△				X			X						X	△		△	△
Vital signs	△	△	△				X			X						X	△		△	△	△				X			X						X	△		△	△
12-lead ECG	△		△				X			X						X	△		△	△	△				X			X						X	△		△	△
Laboratory tests	△	△																	△	△																	△	△
Adverse events			←																→	←																		
Concomitant medications																			→																			→
PK Blood sampling for edaravone			△	X	X	X	X	X	X	X		X	X	X		X	△	X	△	△	X	X	X	X	X	X	X	X		X	X	X		X	△	X	△	

△: To be performed before dosing of edaravone or in the fasting state before breakfast.

○: Subjects will take a high-fat diet over 15 minutes.

n) Subjects will begin taking a meal after completing the prespecified blood sampling for PK and tests.

o) Height will be measured at screening only. Body weight will be measured at screening, hospitalization, and end-of-study assessments. BMI will be calculated at screening and hospitalization assessment.

p) To be performed only in Caucasian subjects.

q) At the time of withdrawal, the same tests will be performed as those of the end-of-study assessment.

3) Group 5: (Period I [Start of the meal 1 hour after dosing], Period II [Dosing 4 hours after the meal], and Period III [No breakfast after dosing])

	Informed consent	Screening	Hospitalization																	Hospitalization																	
				Period I (Start a meal 1 hour after dosing)																Period II (Dosing 4 hours after completion of a meal)																	
Day (time window)	Day of obtaining informed consent	Day -30 to -2	-1	1														2		3	4														5		6	
Time		Visit	Admission	8	8:05	8:15	8:30	9	9:30	10	12	13:30	14	16	18	19	20	8	18	8	6:45	11	11:05	11:15	11:30	12	12:30	13	15	17	19	20	21	23	11	23	8:30	11
Time (h) after dosing				0	0.083	0.25	0.5	1	1.5	2	4	5.5	6	8	10	11	12	24	36	48	-4.25	0	0.083	0.25	0.5	1	1.5	2	4	6	8	9	10	12	24	36	45.5	48
Screening		Δ																																				
Written informed consent	X																																					
Subject characteristics		Δ																																				
Eligibility assessment		Δ	Δ	Δ																																		
Dosing of edaravone				X																		X																
Meal on the days of dosing[a)]								O				X				X					O								X			X						
Height, weight, BMI[b)]		Δ	Δ																																			
Physical examination		Δ	Δ	Δ				X			X						X	Δ		Δ		Δ				X			X					X	X			X
Vital signs		Δ	Δ	Δ				X			X						X	Δ		Δ		Δ				X			X					X	X			X
12-lead ECG		Δ	Δ	Δ				X			X						X	Δ		Δ		Δ				X			X					X	X			X
Laboratory tests		Δ	Δ																	Δ																	Δ	
Adverse events				←																																		→
Concomitant medications																																						→
PK Blood sampling for edaravone				Δ	X	X	X	X	X	X	X		X	X	X		X	Δ	X	Δ		Δ	X	X	X	X	X	X	X	X	X		X	X	X	X		X

Δ: To be performed before dosing of edaravone or in the fasting state before breakfast.

O: Subjects will take a high-fat diet over 15 minutes.

r) Subjects will begin taking a meal after completing the prespecified blood sampling for PK and tests.

s) Height will be measured at screening only. Body weight will be measured at screening, hospitalization, and end-of-study assessments. BMI will be calculated at screening and hospitalization assessment.

t) To be performed only in Caucasian subjects.

u) At the time of withdrawal, the same tests will be performed as those of the end-of-study assessment.

Day (time window)	Screening		Hospitalization																End-of-study assessment[d]
			Period III (No breakfast after dosing)																
	Day -24 to 5	6	7													8		9	14 (±2)
Time	Visit[c]	Admission[c]	8	8:05	8:15	8:30	9	9:30	10	12	12:30	14	16	18	19	20	8	18	8	Visit
Time (h) after dosing			0	0.083	0.25	0.5	1	1.5	2	4	4.5	6	8	10	11	12	24	36	48	
Screening	△																			
Written informed consent																				
Subject characteristics	△																			
Eligibility assessment	△	△	△[c]																	
Dosing of edaravone			X																	
Meal on the days of dosing[a]											X				X					
Height, weight, BMI[b]	△	△																		△
Physical examination	△	△	△				X			X						X	△		△	△
Vital signs	△	△	△				X			X						X	△		△	△
12-lead ECG	△	△	△				X			X						X	△		△	△
Laboratory tests	△	△																	△	△
Adverse events			←																	→
Concomitant medications			—																	→
PK Blood sampling for edaravone			△	X	X	X	X	X	X	X		X	X	X		X	△	X	△	

△: To be performed before dosing of edaravone or in the fasting state before breakfast.

○: Subjects will take a high-fat diet over 15 minutes.

v) Subjects will begin taking a meal after completing the prespecified blood sampling for PK and tests.

w) Height will be measured at screening only. Body weight will be measured at screening, hospitalization, and end-of-study assessments. BMI will be calculated at screening and hospitalization assessment.

x) To be performed only in Caucasian subjects.

At the time of withdrawal, the same tests will be performed as those of the end-of-study assessment.

3. STUDY OBJECTIVE(S) AND ENDPOINTS

3.1 Study Objective(s)

Cohort 1: Drug interaction study

Primary objective: To evaluate the drug interactions, safety, and tolerability of sildenafil, rosuvastatin, or furosemide when coadministered with oral edaravone in healthy adult males.

Secondary objective: To evaluate the pharmacokinetics, safety, and tolerability of oral edaravone.

Cohort 2: Preliminary regimen-finding study

Primary objective: To evaluate the effect of food on the pharmacokinetics of oral edaravone in healthy adult males.

Secondary objective: To evaluate the pharmacokinetics, safety, and tolerability of oral edaravone as well as to evaluate effects of racial difference on edaravone pharmacokinetics.

3.2 Safety Assessments

- Adverse events (AEs) and Adverse Drug Reaction(ADRs)
- 12 lead electrocardiogram (ECG)
- Laboratory tests
- Vital signs
- Sensory tests

3.3 Pharmacokinetics Endpoint(s)/Evaluation(s)

The pharmacokinetic parameters in plasma will be determined from the individual concentration vs. time data by non-compartmental analysis using WinNonlin® professional version 6.3 or later. The exact sampling time (in hours rounded to 3 decimal places) relative to dosing will be used in calculation. For the calculation of PK parameters concentration below the limit of quantification (BLQ) will be imputed with a value of zero.

3.3.1 Assessments of the Effect on Pharmacokinetics

- AUC_{0-24}, $AUC_{0-\infty}$ and C_{max} of rosuvastatin, sildenafil, or furosemide alone and in the presence of edaravone
- AUC_{0-24}, $AUC_{0-\infty}$ and C_{max} of unchanged edaravone, sulfate conjugate, and glucuronide conjugate in fasted condition, 1 hour before meal, and 4 hours after meal
- AUC_{0-24}, $AUC_{0-\infty}$ and C_{max} of unchanged edaravone, sulfate conjugate, and glucuronide conjugate in Japanese and Caucasian

3.3.2 Analysis of Individual Plasma Concentrations and Pharmacokinetic Parameters

The pharmacokinetic concentrations over time (individual and mean values) will be assessed for rosuvastatin, sildenafil, or furosemide alone and in presence of edaravone and for edaravone alone and edaravone in the presence of each victim drug (cohort 1); and for unchanged edaravone, sulfate conjugate, glucuronide conjugate in each meal condition and each race (cohort 2).

Following PK parameters will be calculated for each subject and for each treatment:

- In cohort 1, for rosuvastatin, sildenafil, and furosemide alone and in presence of edaravone: AUC_{0-t}, AUC_{0-24}, $AUC_{0-\infty}$, C_{max}, t_{max}, $t_{1/2}$, Kel, MRT, CL/F, V_z/F, V_{ss}/F
- In cohort 1, for unchanged edaravone, sulfate conjugate (group 2), and glucuronide conugate (group 2) after administration of edaravone alone and in presence of rosuvastatin, sildenafil, or furosemide: AUC_{0-t}, AUC_{0-24}, $AUC_{0-\infty}$, C_{max}, t_{max}, $t_{1/2}$, Kel, MRT*, CL/F*, V_z/F*, V_{ss}/F* (* only for unchanged edaravone)
- In cohort 2, for unchanged edaravone, sulfate conjugate, and glucuronide conugate after administration of edaravone in fasted condition, 1 hour before meal, and 4 hours after meal; or in Japanese anad Caucasian: AUC_{0-t}, AUC_{0-24}, $AUC_{0-\infty}$, C_{max}, t_{max}, $t_{1/2}$, Kel, MRT*, CL/F*, V_z/F*, V_{ss}/F* (* only for unchanged edaravone)

3.4 Other Endpoint(s)/Evaluation(s)

Not applicable in this study.

4. PLANNED ANALYSES

4.1 Interim Analysis

No interim analysis will be performed for this study.

4.2 Final Analysis

Final analysis will be done after database lock as per this SAP. Tables, figures and listings (TFLs) will be prepared by TIT. Acceptance check for the TFLs will be performed by MTPC Data Science Department.

5. ANALYSIS POPULATION(S)

The definitions of the analysis sets are provided below. Safety analysis will be performed in the Safety Analysis Set (SAF) and PK analysis will be performed in the PK Analysis Set (PKPOP).

PK Analysis Set (PKPOP):

- All subjects who received at least 1 dose of the study drug or victim drug and had evaluable PK data.

Safety Analysis Set (SAF):

- All subjects who received at least 1 dose of the study drug or victim drug.

6. GENERAL CONSIDERATIONS

In general, continuous variables will be summarised descriptively using the number of observations, mean, standard deviation, median, minimum and maximum. Categorical variables will be summarised using frequency counts and percentages. The denominator for the percentages will be the total number of subjects in the treatment group and analysis set being presented, unless otherwise specified (e.g. on some occasions, percentages maybe calculated out of the total number of subjects with available data at a particular visit and/or time point).

Unless otherwise specified, baseline for vital signs, physical examination and ECG parameters will be the pre-dose value on Day 1.The baseline for laboratory parameters will be the value at Day -1. The baseline for sensory test will be the value at Day 6 in Group 1 and at Day 3 in Group 2.

For numerical variables, change from baseline will be calculated as the post-baseline value minus the baseline value. If baseline value cannot be determined for a particular variable, the change from baseline will not be calculated.

All data will be listed. Listings will include treatment, scheduled, unscheduled, retest and early discontinuation data. Unscheduled visits and retests (same visit number assigned), will not be displayed in by-visit summary tables.

All medical history and adverse events will be coded from the actual term using the Medical Dictionary for Regulatory Activities (MedDRA) version 21.0.

All prior and concomitant medications will be coded using the World Health Organization (WHO) drug dictionary (WHO DRUG Enhanced Sep-2018 B3).

No analysis visit window will be performed for safety evaluation.

For Caucasian, analysis visits for non-PK measurements are as follows.

CRF visit in Group 3	CRF visit in Group 4	CRF visit in Group 5	Analysis Visit
Screening	Screening	Screening	Screening
Day -1	Day 3	Day 6	Day -1
Day 3	Day 6	Day 9	Day 3

Follow-up	Follow-up	Follow-up	Follow-up

Analysis time windows for blood sampling for PK measurements of each analyte are as follows.

Edaravone, Sulfate Conjugate, Glucuronide Conjugate	**Cohort1 Group1**
Pre-dose on Day 6	Before the first dosing of edaravone
0.083 h after dosing (Day 6, 9, 12)	Scheduled time ± 1 minute
0.25, 0.5, 1, 1.5, 2, 4, 6, 8, 12 h after dosing (Day 6, 9, 12)	Scheduled time ± 5 minutes
Pre-dose on Day 7–13	Scheduled time - 15 minutes
24 h after dosing (Day 14)	Scheduled time ± 15 minutes

Edaravone, Sulfate Conjugate, Glucuronide Conjugate	**Cohort1 Group2**
Pre-dose on Day 3	Before the first dosing of edaravone
0.083h after dosing (Day 3, 6)	Scheduled time ± 1 minute
0.25, 0.5, 1, 1.5, 2, 4, 6, 8, 12h after dosing (Day 3, 6)	Scheduled time ± 5 minutes
Pre-dose on Day 4–7	Scheduled time - 15 minutes
24h after dosing (Day 8)	Scheduled time ± 15 minutes

Edaravone, Sulfate Conjugate, Glucuronide Conjugate	**Cohort2**
Pre-dose on Day 1, 4, 7	Before dosing of edaravone
0.083 h after dosing (Day 1, 4, 7)	Scheduled time ± 1 minutes
0.25, 0.5, 1, 1.5, 2, 4, 6, 8, 10, 12h after dosing (Day 1, 4, 7)	Scheduled time ± 5 minutes
24, 36h after dosing (Day 2, 5, 8)	Scheduled time ± 15 minutes
48h after dosing (Day 3, 6, 9)	Scheduled time ± 15 minutes

Rosuvastatin	**Cohort1 Group1**
Pre-dose on Day 1, 9	Before dosing of Rosuvastatin
0.5, 1, 2, 3, 4, 6, 8, 12h after dosing (Day 1, 9)	Scheduled time ± 5 minutes
16, 24h after dosing (Day 2, 10)	Scheduled time ± 15 minutes
48h after dosing (Day 3, 11)	Scheduled time ± 15 minutes

Sildenafil	**Cohodrt1 Group1**
Pre-dose on Day 4, 12	Before dogin of Sildenafil
0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12h after dosing (Day 4, 12)	Scheduled time ± 5 minutes
24h after dosing (Day 5, 13)	Scheduled time ± 15 minutes
48h after dosing (Day 6, 14)	Scheduled time ± 15 minutes

Furosemide	**Cohort1 Group2**
Pre-dose on Day 1, 6	Before dosing of Furosemide
0.5, 1, 1.5, 2, 3, 4, 6, 8, 12h after dosing (Day 1, 6)	Scheduled time ± 5 minutes
24h after dosing (Day 2, 7)	Scheduled time ± 15 minutes
48h after dosing (Day 3, 8)	Scheduled time ± 15 minutes

7. STATISTICAL METHODOLOGY

7.1 Statistical Considerations

Prior to database lock, a data review meeting (DRM) was conducted. Protocol deviations, protocol defined analysis populations was confirmed during the meeting. Additional data handling rules was introduced as results of data review.

Only valid PK data will be included in the summary tables or figures. PK data that are considered "invalid" or "abnormal" will be flagged in the listing. The PK data handling will be assessed during DRM prior to database lock.

7.2 Data Handling

All data will be used to their maximum possible extent without any imputations for missing data, i.e. missing values will not be replaced or estimated. Detailed data handling was determined at DRM, with justified rationale.

The 1st DRM was conducted at 20 March 2019 prior to database lock. As results of data review, "J02128" was excluded from safety analysis because the subject dropped out before taking the study drug. Some PK data were excluded from PK analysis because they could not be measured properly due to insufficient volume of plasma sample.

After the 1st DRM, the definition of SAF and PKPOP was changed. Due to the change, the 2nd DRM was conducted prior to database lock.

As results of the 2nd DRM, no subject was excluded from safety analysis and PK analysis.

7.3 Sample Size and Power Considerations

- A total of 84 subjects;

  Cohort 1: 66 subjects(Group 1: 32 subjects, Group 2: 34 subjects)

  Cohort 2: 18 subjects(Japanese 9 subjects, Caucasian 9 subjects)

[Rationales for setting]

(1) Cohort 1

Because there is no information about pharmacokinetic parameters for coadministration of each victim drug (rosuvastatin, sildenafil, or furosemide) and edaravone, the number of subjects for each group was set as follows based on data about C_{max} and AUC after administration of victim drugs obtained from previous studies and literatures.

- Group 1

  Regarding rosuvastatin, based on results of previous studies, geometric CV (intra-

individual) of C_{max} and $AUC_{0-\infty}$ was hypothesized to be 21.0% and 23.0%, respectively. On the assumption that when only rosuvastatin is administered and when it is coadministered with edaravone, ratio of both parameters would be 1.00; the number of subjects was calculated to be 20 which would allow power of at least 80% in 2 one-tailed tests with significance level of 5%.

Regarding sildenafil, based on results of previous studies, geometric CV (intra-individual) of C_{max} and $AUC_{0-\infty}$ was hypothesized to be 28.9% and 22.8%, respectively. On the assumption that when only sildenafil is administered and when it is coadministered with edaravone, ratio of both parameters would be 1.00; the number of subjects was calculated to be 29 which would allow power of at least 80% in 2 one-tailed tests with significance level of 5%.

Based on the above results, the target number of subjects was set at 32, which is the total of 3 (an anticipated number of subjects who might withdraw from the study) and 29 (the number of subjects calculated based on sildenafil data).

- Group 2

Based on results of previous studies, intra-individual geometric CV of C_{max} and $AUC_{0-\infty}$ of furosemide was hypothesized to be 29.5% and 11.5%, respectively. On the assumption that when only furosemide is administered and when it is coadministered with edaravone, ratio of both parameters would be 1.00; the number of subjects was calculated to be 31 which would allow power of at least 80% in 2 one-tailed tests with significance level of 5%. Based on the above results, the target number of subjects was set at 34 on the basis that 3 subjects might withdraw from the study.

Based on the above, the target number of subjects was set at 66 for Cohort 1 (32 for Group 1, 34 for Group 2).

(2) Cohort 2

The target number of subjects was set on the assumption that it would allow obtaining results that will meet the study objectives although it is not based on statistical calculations.

7.4 Disposition of Subjects

The number and percentage of subjects who completed the study will be presented. Subjects who withdraw will be summarized by reasons for withdrawn.

The number and percent of subjects in each protocol defined analysis set will be presented.

Subject disposition will be listed for all enrolled subjects. Participation in each defined population will be listed.

Whether or not inclusion/exclusion criteria met will also be listed.

7.5 Demographic and Other Baseline Characteristics

All demographic data and other baseline characteristics (sex, age, height(cm), weight(kg), BMI, race, medical history, complications, concomitant medications, allergic history (including drug allergies), drinking status, smoking status) will be summarized on the SAF.

A listing of all demographic data and baseline characteristics will be presented for all enrolled subjects.

(1) BMI

BMI = Body weight (kg) / height $(m)^2$ (rounded to one decimal place)

Height will be the value obtained at screening.

Body weight will be the value obtained at the time of hospitalization.

7.6 Medical History and Complications

All medical history data and complications data will be listed for all enrolled subjects.

7.7 Prior or Concomitant Medications

All prior or concomitant medications data will be listed by subject including the ATC level 2 and preferred term.

Prior medications are medications which stopped prior to the first dose of study drug or victim drug.

Concomitant medications are medications which started prior to, on or after the first dose of study medication and ended on or after the date of first dose of study drug or victim drug, or were on-going at the end of the study.

7.8 Study Medication Exposure

All study drug and victim drug administration data will be listed.

7.9 Pharmacokinetic Assessments

All pharmacokinetic data will be listed on the PKPOP, whether or not the subject has completed all treatment periods. Summaries of concentrations, PK parameters and statistical analysis for rosuvastatin, sildenafil, furosemide, and edaravone and its metabolites will be performed using data from subjects whose PK data will be available in at least one treatment period (e.g. rosuvastatin alone or rosuvastatin + edaravone; edaravone in fasted condition, 1 hour before meal, or 4 hours after meal).

The PK parameters determined by non-compartmental analysis will be listed for the PKPOP. The PK parameters will be calculated using the actual times after dosing (in hours rounded to 3 DP) rather than the nominal times using the reported concentration values. For the

calculation of PK parameters concentration below the limit of quantification (BLQ) will be imputed with a value of zero. All PK parameters will be included in the data listings.

For calculation of AUCs, which is for PK, missing data will be treated as if the respective sample never had been scheduled for the calculation by the linear-linear trapezoidal rule; this has the same effect as if the missing value had been estimated by linear interpolation.

All summaries will be done on the PKPOP and will include the following statistics:

- number of subjects [N]
- number of observations [n]
- Mean
- Standard Deviation [SD]
- Median, Minimum and Maximum
- CV%, where CV% = (SD/mean) x 100
- Geometric mean
- Geometric CV%, where Geometric CV% = $[\exp(\text{sigma}^2) - 1]^{1/2} \times 100$, where sigma represents the SD computed on the logarithmic transformed concentration.

For the calculation of the summary statistics for plasma concentrations, concentration values reported as BLQ will be set to zero. For calculation of geometric mean and geometric CV%, LLOQ/2 will be used for values BLQ.

7.9.1 Analysis of Individual Plasma Concentrations and Pharmacokinetic Parameters for rosuvastatin, sildenafil and furosemide

Plasma Concentration

Plasma concentration data for each victim drug (alone) and each victim drug in presence of edaravone will be listed by subject, treatment and nominal time point and summarised by treatment.

Individual and mean plasma concentration versus time curves will be plotted on both linear/linear and log/linear scales separately for rosuvastatin, sildenafil and furosemide. For individual plots actual time will be used. There will be two sets of individual plots: a) separate plots for each treatment (victim drug alone and victim drug + edaravone) with all subjects included and b) with separate plots for each subject with both treatments included.

PK Parameters

The following PK parameters for rosuvastatin, sildenafil, and furosemide will be calculated from measured plasma concentrations: AUC_{0-t}, AUC_{0-24}, $AUC_{0-\infty}$, C_{max}, t_{max}, $t_{1/2}$, Kel, MRT, CL/F, V_z/F, V_{ss}/F

All PK parameters will be summarised by analyte alone and in combination with edaravone.

Theses parameters will also be listed. For all PK profiles for which the Kel has been calculated, the following will be listed:

- Number of data points used in the regression analysis for estimation of Kel.

- The lower and upper limits of the time period spanned by the data points used in the regression analysis for estimation of Kel.
- Adjusted R-squared value.
- Percentage of $AUC_{0-\infty}$ extrapolated.

For C_{max}, AUC_{0-t}, AUC_{0-24} and $AUC_{0-\infty}$ spaghetti plots will be produced showing individual changes from victim drug alone to victim drug + edaravone.

For C_{max}, AUC_{0-t}, AUC_{0-24} and $AUC_{0-\infty}$ ratios (victim drug + edaravone / victim drug alone) will be calculated for each subject. These will be listed and summary statistics (N, n, mean, SD, median, minimum, maximum) for these will also be presented.

C_{max}, AUC_{0-t}, AUC_{0-24} and $AUC_{0-\infty}$ will be analysed as for the assessments of effect on pharmacokinetics of rosuvastatin, sildenafil, and furosemide by edaravone as explained in 7.9.3, but 90% CI for the ratio of AUC_{0-t} and AUC_{0-24} will not be used for the assessment of the effect of edaravone on rosuvastatin, sildenafil and furosemide.

7.9.2 Analysis of Individual Plasma Concentrations and Pharmacokinetic Parameters for Unchanged Edaravone, Sulfate Conjugate, and Glucuronide Conjugate (Cohort 1)

Plasma Concentration

Plasma concentration data of unchanged edaravone, sulfate conjugate (group 2), and glucuronide conjugate (group 2) after administration of edaravone (alone) and in presence of each victim drug will be listed by subject, treatment and nominal time point.

Summary statistics (N, n, mean, SD, minimum, median, maximum, CV%, geometric mean and geometric CV%) will be calculated for plasma concentrations of each analyte at each time point by treatment. Plasma concentration data of unchanged edaravone after administration of edaravone alone will be summarized in each group and in combined groups.

Individual and mean plasma concentration versus time curves will be plotted on linear/linear scale for each analyte using actual and scheduled times respectively.

Concentration at post-24 h

Concentrations at post-24 hours of unchanged edaravone, sulfate conjugate (group 2) and glucuronide conjugate (group 2) are the pre-dose concentrations for each day during Day 6 to Day 13 (group 1) or Day 3 to Day 7 (group 2).

The ratio of concentrations at post-24 h on each day (Day 6 to Day 12 for group 1 or Day 3 to Day 6 for group 2) compared to the concentration at post-24 h on the last dosing day (Day 13 for group 1 and Day 7 for group 2) will be calculated using observed values (i.e., the data will not be log-transformed for calculation of the ratios). In the event that the pre-dose concentration is BLQ, the ratio will not be calculated for that day (in this case the result will be shown as not calculated [NC] in the listings and will be excluded from the summary statistics). The ratios on each day (Day 6 to Day 12 or Day 3 to Day 6) compared to the last dosing day will also be listed. The ratios each day (e.g., Day 7/Day 13) will be summarized using descriptive statistics.

PK Parameters

The following PK parameters for unchanged edaravone, sulfate conjugate (group 2) and glucuronide conjugate (group 2) will be calculated from measured plasma concentrations: AUC_{0-t}, AUC_{0-24}, $AUC_{0-\infty}$, C_{max}, t_{max}, $t_{1/2}$, Kel, MRT*, CL/F*, V_z/F*, V_{ss}/F* (* only for unchanged edaravone)

The PK parameters will be listed. Summary statistics (N, n, mean, SD, median, minimum, maximum, geometric mean and geometric CV%) will be presented for all PK parameters by treatment (edaravone alone or in combination with each victim drug). The PK parameters of unchanged edaravone after administration of edaravone alone will be summarized in each group and in combined groups.

Theses parameters will also be listed. For all PK profiles for which the Kel has been calculated, the following will be listed:

- Number of data points used in the regression analysis for estimation of Kel.
- The lower and upper limits of the time period spanned by the data points used in the regression analysis for estimation of Kel.
- Adjusted R-squared value.
- Percentage of $AUC_{0-\infty}$ extrapolated.

7.9.3 Assessments of effect on pharmacokinetics of rosuvastatin, sildenafil, and furosemide by edaravone

PK parameters $AUC_{0-\infty}$ and C_{max} of rosuvastatin, sildenafil, and furosemide alone and in the presence of edaravone will be analysed for the assessments of effect on pharmacokinetics of rosuvastatin, sildenafil, and furosemide by edaravone.

Descriptive Statistics:

Pharmacokinetic parameters will be summarized by analyte (i.e. Rosuvastatin, Sildenafil and Furosemide) alone and in presence of edaravone.

Statistical Analysis:

A linear mixed effects model will be fitted to log-transformed PK parameters (AUC_{0-t}, AUC_{0-24}, $AUC_{0-\infty}$, and C_{max}) including treatment as fixed effect and subject as random effect. Treatment will be victim drug + edaravone or victim drug alone. Estimates of mean difference between treatments will be obtained with 90% CI for the difference. These estimates and limits will then be back-transformed to obtain ratios of least squares geometric means A 90% CI for the ratio of $AUC_{0-\infty}$ as well as C_{max} which lie entirely within the limits of 0.80 to 1.25 provides evidence of no effect of edaravone on rosuvastatin, sildenafil and furosemide.

7.9.4 Analysis of Individual Plasma Concentrations and Pharmacokinetic Parameters for Unchanged Edaravone, Sulfate Conjugate, and GlucuronideConjugate (Cohort 2)

Plasma Concentration

Plasma concentration data of unchanged edaravone, sulfate conjugate, and glucuronide conjugate after administration of edaravone in each meal condition and each race will be listed by subject, treatment and nominal time point.

Summary statistics (N, n, mean, SD, minimum, median, maximum, CV%, geometric mean and geometric CV%) will be calculated for plasma concentrations of each analyte at each time point by treatment.

Individual and mean plasma concentration versus time curves will be plotted on both linear/linear and log/linear scales separately for unchanged edaravone, sulfate conjugate, and glucuronide conjugate. For individual plots actual time will be used. There will be two sets of individual plots: a) separate plots for each meal condition (fasted condition, 1 hour before meal, and 4 hours after meal) and each race (Japanese and Caucasian) with all subjects included and b) with separate plots for each subject with all meal conditions included.

PK Parameters

The following PK parameters for unchanged edaravone, sulfate conjugate and glucuronide conjugate will be calculated from measured plasma concentrations: AUC_{0-t}, AUC_{0-24}, $AUC_{0-\infty}$, C_{max}, t_{max}, $t_{1/2}$, Kel, MRT*, CL/F*, V_z/F*, V_{ss}/F* (* only for unchanged edaravone)

The PK parameters will be listed. Summary statistics (N, n, mean, SD, median, minimum, maximum, geometric mean and geometric CV%) will be presented for all PK parameters by treatment (edaravone in fasted condition, or before or after meal) and race (Japanese or Caucasian).

Theses parameters will also be listed. For all PK profiles for which the Kel has been calculated, the following will be listed:

- Number of data points used in the regression analysis for estimation of Kel.
- The lower and upper limits of the time period spanned by the data points used in the regression analysis for estimation of Kel.
- Adjusted R-squared value.
- Percentage of $AUC_{0-\infty}$ extrapolated.

For C_{max}, AUC_{0-t}, AUC_{0-24} and $AUC_{0-\infty}$ spaghetti plots or scatter plots will be produced showing individual differences between fasted condition and administration before or after meal, and showing differences in parameter distributions of Japanese and Caucasian subjects.

For C_{max}, AUC_{0-t}, AUC_{0-24} and $AUC_{0-\infty}$ ratios (administration before or after meal/ fasted condition in Japanese subjects) will be calculated for each subject. These will be listed and summary statistics (N, n, mean, SD, median, minimum, maximum) for these will also be presented.

7.9.5 Assessments of effect of meal conditions on pharmacokinetics of Unchanged Edaravone, Sulfate Conjugate, and Glucuronide Conjugate

PK parameters $AUC_{0-\infty}$ and C_{max} of unchanged edaravone, sulfate conjugate, and glucuronide conjugate for each meal condition will be analysed for the assessments of effect on pharmacokinetics of unchanged edaravone, sulfate conjugate, and glucuronide conjugate by meal conditions.

Descriptive Statistics:

Pharmacokinetic parameters will be summarized by analyte (i.e. unchanged edaravone, sulfate conjugate, and glucuronide conjugate) in fasted condition, 1 hour before meal, and 4 hours after meal.

Statistical Analysis:

A linear mixed effects model will be fitted to log-transformed PK parameters (AUC_{0-t}, AUC_{0-24}, $AUC_{0-\infty}$, and C_{max}) including meal condition, period and group as fixed effect and subject as random effect. Meal condition will be in fasted condition, 1 hour before meal or 4 hours after meal. Estimates of mean difference between fasted condition and before or after meals will be obtained with 90% CI for the difference. These estimates and limits will then be back-transformed to obtain ratios of least squares geometric means A 90% CI for the ratio of $AUC_{0-\infty}$ as well as C_{max} which lie entirely within the limits of 0.80 to 1.25 provides evidence of no effect of food intake on unchanged edaravone, sulfate conjugate, and glucuronide conjugate.

7.9.6 Assessments of race on pharmacokinetics of Unchanged Edaravone, Sulfate Conjugate, and Glucuronide Conjugate

PK parameters $AUC_{0-\infty}$ and C_{max} of unchanged edaravone, sulfate conjugate, and glucuronide conjugate in each race will be analysed for the assessments of effect on pharmacokinetics of unchanged edaravone, sulfate conjugate, and glucuronide conjugate by race.

Descriptive Statistics:

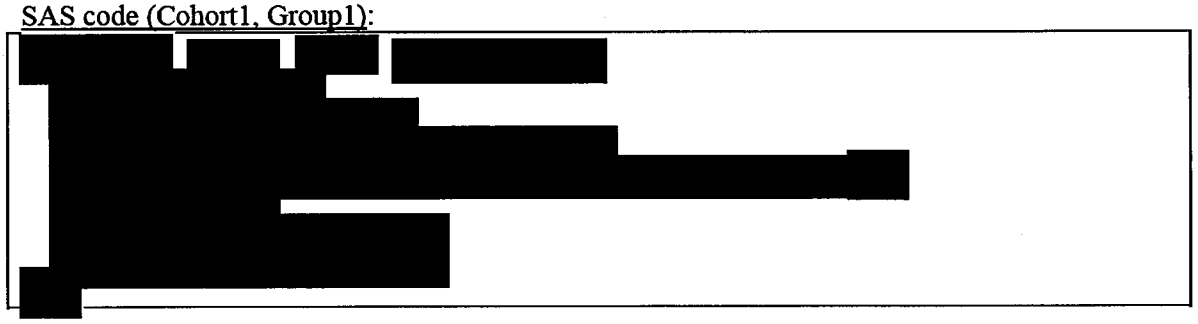
Pharmacokinetic parameters will be summarized by analyte (i.e. unchanged edaravone, sulfate conjugate, and glucuronide conjugate) in Japanese and Caucasian.

Statistical Analysis:

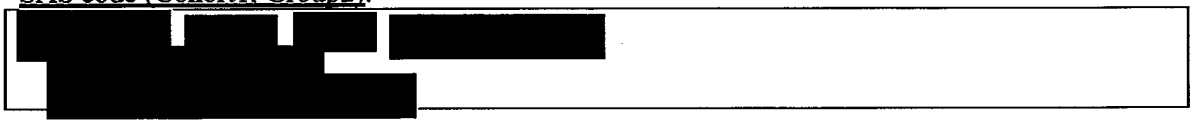
A linear model will be fitted to log-transformed PK parameters (AUC_{0-t}, AUC_{0-24}, $AUC_{0-\infty}$, and C_{max}) including race as fixed effect. Race will be Japanese or Caucasian. Estimates of mean difference between Japanese and Caucasian will be obtained with 90% CI for the difference. These estimates and limits will then be back-transformed to obtain ratios of least squares geometric means A 90% CI for the ratio of $AUC_{0-\infty}$ as well as C_{max} which lie entirely within the limits of 0.80 to 1.25 provides evidence of no effect of race on edaravone, sulfate conjugate, and glucuronide conjugate.

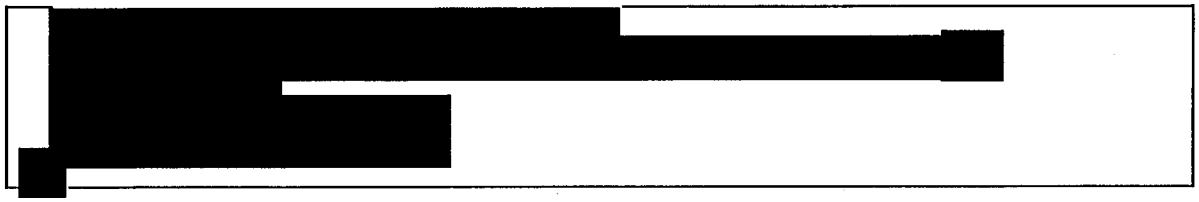
- Example Code

SAS code (Cohort1, Group1):

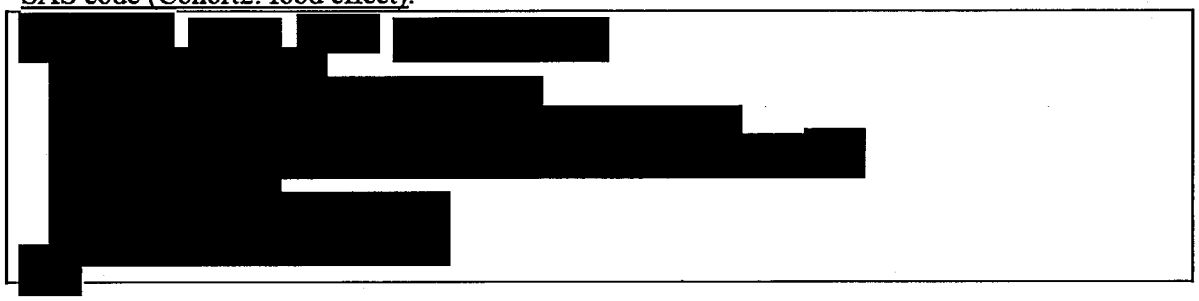


SAS code (Cohort1, Group2):

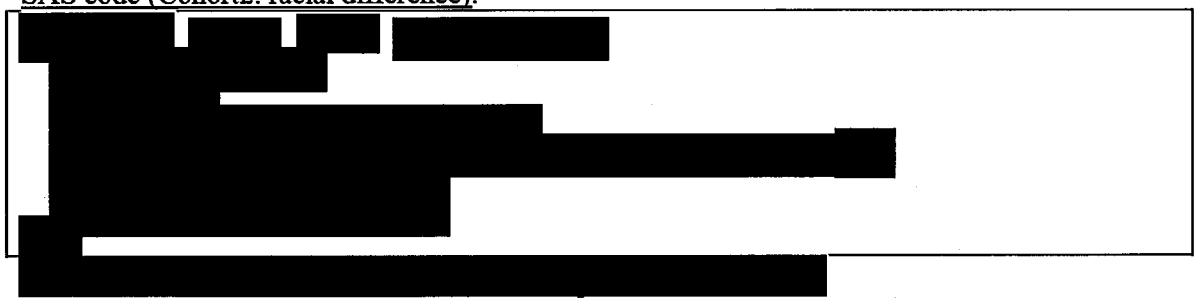




SAS code (Cohort2: food effect):



SAS code (Cohort2: racial difference):



7.10 Safety Assessments

All safety and tolerability variables will be listed and summarised descriptively or frequency tables where appropriate on the SAF. No imputation will be made in case of missing values.

7.10.1 Adverse Events

Adverse events (AEs) will be assigned to a period based on the start date. ADRs are defined as AEs that determined to have a "Reasonable Possibility" of causal relationship to the study drug.

Duration of the AE and time to the AE occurrence from start of study drug or victim drug will be calculated and presented in days and time.

AE Occurrence from Start of Study Drug or Victim Drug = Date/Time of Onset- Start Date/Time of Administration. If Time is Missing, Date of Onset - Date of Administration +1. Duration of AE = Date/Time of Resolution - Date/Time of Onset. If Time is Missing, Date of Resolution - Date of Onset +1. The following summaries of AEs will be presented.

- Overall summary of AEs.
- Summary of AEs by SOC and PT.
- Summary of ADRs by SOC, PT
- Summary of AEs by SOC, PT by maximum severity

The frequency and incidence of AEs will be summarised by System Organ Class (SOC) and Preferred Term (PT) by cohort, group and treatment. The summary will be sorted by International Agreed Order for SOC and alphabetical order for PT (or by frequency from the highest to the lowest).

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

Treatment periods for adverse events are as followed.

① Group 1

Visit	Treatment
Day 1 ~ Day 4	Rosuvastatin alone
Day 4 ~ Day 5	Sildenafil alone
Day 6 ~ Day 8	Edaravone alone
Day 9 ~ Day 11	Rosuvastatin + Edaravone
Day 12 ~ Day 20 including end of study assessment	Sildenafil + Edaravone

② Group 2

Visit	Treatment
Day 1 ~ Day 2	Furosemide alone
Day 3 ~ Day 5	Edaravone alone
Day 6 ~ Day 14 including end of study assessment	Furosemide+ Edaravone

③ Japanese in Group 3, Group 4 and Group 5

Visit in Group 3	Visit in Group 4	Visit in Group 5	Treatment
Day 1 ~ Day 3	Day 7 ~ Day 9 including end of study assessment	Day 4 ~ Day 6	Fasted
Day 4 ~ Day 6	Day 1 ~ Day 3	Day 7 ~ Day 9 including end of study assessment	Before meal
Day 7 ~ Day 9 including end of study assessment	Day 4 ~ Day 6	Day 1 ~ Day 3	After meal

④ Caucasian in Group 3, Group 4 and Group 5

Visit in Group 3	Visit in Group 4	Visit in Group 5	Treatment
Day 1 ~ Day 8 including end of study assessment	Day 4 ~ Day 11 including end of study assessment	Day 7 ~ Day 14 including end of study assessment	Fasted

7.10.2 Laboratory Tests

Clinical significance of 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). A listing of laboratory values will be provided for subjects with any clinical significant findings (list relevant laboratory parameters only).

Lab parameter values and changes from baseline, except for urinalysis will be listed and summarized descriptively by cohort, group and scheduled visit.

For urinalysis, number and percentage will be presented. Shift tables will present the changes in clinically relevant categories from baseline to each scheduled post-baseline visit.

Below is a list of the laboratory tests:

Hematology	Hemoglobin, hematocrit, red blood cell count, white blood cell count, platelet count, MCH, MCHC, MCV, differential white blood count
Biochemistry	Na, K, Cl, Ca, inorganic phosphorus, urea nitrogen, creatinine, uric acid, total bilirubin, direct bilirubin, ALT, AST, γ-GTP, ALP, LDH, CK, amylase, total cholesterol, triglycerides, LDL-C, HDL-C, total protein, albumin, glucose
Coagulation test	Prothrombin time, activated partial thromboplastin time
Urinalysis	Qualitative tests (pH, specific gravity, protein, glucose, occult blood, urobilinogen, bilirubin, ketones)

Any BLQ data will be treated as 0 in summary statistics.

7.10.3 Vital Signs

All vital sign data will be listed.

Vital signs (systolic blood pressure, diastolic blood pressure, pulse rate, body temperature) values and changes from baseline will be summarised descriptively by cohort, group and scheduled time point.

7.10.4 12-lead ECGs

All 12-lead ECG parameters and findings (Heart Rate, RR, PR, QRS, QT, QTcF, overall evaluation) will be listed. 12-lead ECG parameter values and changes from baseline will be summarized descriptively by cohort, group and scheduled time point.

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

- QTcF >500msec at timepoint
- 500≥ QTcF >480msec at timepoint
- 480≥ QTcF >450msec at timepoint
- QTcF ≤450msec at timepoint
- Change from baseline in QTcF > 30 msec
- Change from baseline in QTcF > 60 msec

A listing of 12-lead ECG parameter values will be provided for subjects with any clinical significant findings.

7.10.5 Sensory Tests

All sensory tests data will be listed.

Sensory tests (vibratory sensation) values and changes from baseline will be summarized descriptively by group and by visit.

With respect to sensory tests (numbness and staggering) including severity, a shift table of the changes from baseline will be shown by group and by visit.

7.10.6 Physical Examinations

All physical examination data will be listed.

8. CHANGES FROM THE PROTOCOL

The definition of PKPOP and SAF is changed from the protocol. The reason to change the definition of SAF is to enable assessments of safety of any administration regardless of study drug.

The reason to change the definition of PKPOP is to enable assessments of effect on pharmacokinetics of victim drugs by edaravone using subjects whose PK data are available regardless of completion of all treatments (victim drug alone and victim drug + edaravone).

9. DATA NOT SUMMARISED OR PRESENTED

Not applicable in the study.

10. REFERENCES

None.

11. VALIDATIONS

SAS software version 9.4 or later will be used for statistical analysis.

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

12. PROGRAMMING AND DATA PRESENTATION CONVENTIONS

12.1 For Summary Statistics and Safety

- Minimum and maximum will be quoted to the number of decimal places (DP) as recorded for the original (observed) data.
- Means, medians and SD will be quoted to one further DP.

12.2 For Plasma Concentrations

- Individual values, minimum and maximum will be presented with the number of decimal places to which they are reported,
- Summary statistics (mean, SD, median and geometric mean) will be presented with 2 DPs for edaravone and its metabolites, and each victim drug.
- Summary statistics (CV% and geometric mean CV %) will be presented to 1 DP.
- Individual values and summary statistics for ratio of trough concentrations will be presented to 3 DPs.

12.3 For PK Parameters

➢ Individual values and summary statistics will be presented with a number of decimal places as shown in the table below.

➢ Ratios of geometric LS means and CIs will be presented as percentages. Percentages will be presented to two DPs.

Number of Decimal Places for the Presentations of PK Parameters

Statistics	Unit	Individual value, Minimum, Maximum	Mean, SD, Geometric mean	CV%, Geometric CV %
AUC	h•ng/mL	2	2	1
C_{max}	ng/mL	Reported value	2	1
t_{max}	h	2	2	1
Kel	1/h	5	5	1
$t_{1/2}$	h	2	2	1
Vz/F	L	0	0	1
CL/F	L/h	0	0	1
MRT	h	2	2	1
Number of Kel points	–	0	–	–
Lower Limit of Kel	h	2	–	–
Upper Limit of Kel	h	2	–	–
Adjusted R^2	–	2	–	–
$AUC\%_{ex}$	%	2	–	–

AUCs ratio and C_{max} ratio will be presented to 3 DPs.

12.4 Other Specifications

- Column headers in mixed case, with "(N=n)" below period to denote the denominator.
- Categories (i.e. in column 1) in sentence case, in the order on the CRF.
- Ordering of statistics N, Mean, SD, Minimum, Median and Maximum (and labelled as such). CV%, geometric means and geometric CV% also for PK parameters.

13. PHARMACOKINETIC PARAMETER CALCULATIONS

- Actual blood sampling times for the assay of edaravone and its metabolites, rosvastatin, sildenafil, and furosemide will be used in the calculation of pharmacokinetic parameters
- All concentrations below the LLOQ will be set at zero for pharmacokinetic calculations
- When Kel is missing (or cannot be determined), $t_{1/2}$ $AUC_{0-\infty}$, MRT, CL/F and V_z/F, V_{ss}/F will not be calculated.

PK Parameter Calculations		
Parameters	Unit	Calculation
AUC_{0-t}	h·ng/mL	will be calculated using the linear trapezoidal method and actual times $$AUC_{0-t} = \sum_{i=1}^{n} \frac{t_i - t_{i-1}}{2}(C_{i-1} + C_i)$$
AUC_{0-24}	h·ng/mL	will be calculated using time until 24 h drug concentration
$AUC_{0-\infty}$	h·ng/mL	$AUC_{0-\infty} = AUC_{0-t} + \frac{C_{last}}{Kel}$
C_{max}	ng/mL	will be determined using maximum drug concentration
t_{max}	h	will be determined using time to maximum drug concentration
$t_{½}$	h	$t_{½}$ will be determined as: $t_{1/2} = \frac{\log_e(2)}{Kel}$
Kel	1/h	The exponential rate constant of the terminal phase, kel, will be estimated by log-linear regression, if determinable. The number of data points included in the regression will be determined by visual inspection. Wherever possible, a minimum of 3 data points will be used in the estimation of Kel. During the analysis, this calculation method repeats regressions using the last three points with non-zero concentrations, then the last four points, last five, etc. The time of maximum concentration (tmax) will be excluded from the estimation of Kel. Points with a value of zero for the dependent variable are excluded. For each regression, an adjusted R^2 is computed $$\text{Adjusted } R^2 = 1 - \frac{(1 - R^2) + (n - 1)}{(n - 2)}$$ where n is the number of data points in the regression and R^2 is the square of the correlation coefficient. The regression with the largest adjusted R^2 is selected to estimate kel, with these caveats: - If the adjusted R^2 does not improve, but is within

		0.0001 of the largest adjusted R^2 value, the regression with the larger number of points is used. - kel must be positive, and calculated from at least three data points.
MRT	h	$AUMC_{0-\infty} = \sum_{i=1}^{n} \frac{(t_i - t_{i-1})(t_i \times C_i + t_{i-1} \times C_{i-1})}{2} + \frac{t \times C_t}{Kel} + \frac{C_t}{(Kel)^2}$ $MRT_{0-\infty} = \frac{AUMC_{0-\infty}}{AUC_{0-\infty}}$
CL/F	L/h	$CL/F = \frac{Dose}{AUC_{0-\infty}}$
V_z/F	L	$V_z/F = CL/F \times \frac{1}{Kel}$
V_{ss}/F	L	$V_{ss}/F = MRT \times CL/F$
Number of Kel points	–	will be determined using number of points used in computing Kel. If Kel cannot be estimated, zero.
Lower Limit of Kel	h	will be determined using lower limit on time to be included in the calculation of Kel
Upper Limit of Kel	h	will be determined using upper limit on time to be included in the calculation of Kel
$AUC\%_{ex}$	–	$AUC\%_{ex} = \frac{AUC_{0-\infty} - AUC_{0-t}}{AUC_{0-\infty}} \times 100$