STATISTICAL ANALYSIS PLAN

Protocol Title:	RESCUE: A Randomized, Blinded, Placebo-controlled, Parallel Group Design to Determine the Safety of RNS60 in Large Vessel Occlusion Stroke Patients Undergoing Endovascular Thrombectomy
Protocol Number:	06.5.1.H1
Protocol Version/Date:	2.4/May 09, 2022
Investigational Drug:	RNS60
Sponsor:	Revalesio Corporation 1202 East D Street Tacoma, WA 98421
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SIGNATURE PAGE

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We, the undersigned, have reviewed and approved this Statistical Analysis Plan:

Signature	**Date**
[redacted]	14-Nov-2023
[redacted]	14-Nov-2023
[redacted]	14-Nov-2023

SAP VERSION HISTORY

Version	Version Date	Description of Changes
1.0	10 August 2023	Final Signed version
2.0	13 October 2023	• Updated section 2.3.1, item (2) to remove “all-cause” and “stroke-related mortality” from the endpoint description to remain consistent with protocol. 90-day mortality is the primary safety endpoint collected in case report forms. “All-cause” and “stroke-related” mortality categories are not collected separately. • Updated section 3.1.1 to add clarification baseline definition for blood work, ECG, CXR. Baseline definition added for infarct volume. • Updated section 3.3.1 to state that Disposition will be summarized by Enrolled Population. Removed “Screened subjects” as a disposition category because all screened subjects are not recorded in electronic data capture. • Updated section 3.4.2 subsection (1) to include dichotomized mRS counts and percentages and include bar charts for mRS. • Updated section 3.4.2 subsection (2) to include additional details for infarct volume summarization. • Updated section 3.4.2 subsection (3) to include NIHSS spaghetti plots. • Updated section 3.4.2 subsection (4) to designate subjects with worsening of stroke. • Removed previous text under section 3.4.4. Added subsection 3.4.4.1 for analysis of mRS, 3.4.4.2 for correlation analysis of infarction volume and clinical outcomes, and 3.4.4.4 for analyses with additional covariates (post-hoc) . • Updated section 3.5.1 to add “Study drug related TESAEs” as an overview category. TEAE occurrence by month updated to

Version	Version Date	Description of Changes
		display occurrence category by week for the first month and monthly thereafter. • Updated section 3.5.3 to add Table 6 for derived timepoints and Table 7 with vital signs range • Updated section 3.5.5 to include language on derived timepoints. • Updated section 6/4 to clarify primary safety endpoint a s90-day mortality. • Global change: Updated treatment name as RNS60 1.0 mL/kg/h • Included additional reference FDA (2018) in Appendix B.
3.0	14 November 2023	• Section 3.4.2: Replaced “Laplace” method in GLM analysis with RSPL. Updated seed value for imputation analysis of each endpoint. Added clarification for baseline NIHSS and ASPECTS. • Updated section 3.4.2 subsection (3) to add NIHSS analysis at Day 90. • Updated section 3.4.2 subsection (5) for analysis for functional independence using Barthel Index. • Updated section 3.4.4.1 to fix typographical error in calculation of U-Statistic.

PROTOCOL VERSION HISTORY

Version	Version Date	Section(s) Impacted in SAP
Original Protocol/Version 1.0	January 07, 2021	None
Protocol Revision/Version 2.0	January 25, 2021	None
Protocol Revision/Version 2.1	April 14, 2021	None
Protocol Revision/Version 2.2	August 26, 2021	None
Protocol Revision/Version 2.3	January 27, 2022	None
Protocol Revision/Version 2.4	May 09, 2022	None

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LIST OF ABBREVIATIONS

Abbreviation	Definition
ADaM	Analysis Data Model
AIS	Acute Ischemic Stroke
AE	Adverse Event
ANCOVA	Analysis of Covariance
ASPECTS	Alberta Stroke Program Early Computerized Tomography Score
ATC	Anatomical therapeutic chemical
BI	Barthel Index
BP	Blood Pressure
CDISC	Clinical Data Interchange Standards Consortium
CI	Confidence Interval
COVID-19	Coronavirus Disease 2019
CRF	Case Report Form
CRO	Contract Research Organization
CSR	Clinical Study Report
CTA	Computed Tomography Angiography
CVA	Cardiovascular Accident
CXR	Chest X-ray
DWI	Diffusion Weighted Imaging
DSMB	Data Safety Monitoring Board
ECG	Electrocardiogram
EQ-5D-5L	EuroQol health-related quality of life
EVT	Endovascular Thrombectomy
FLAIR	Fluid Attenuated Inversion Recovery
GLM	Generalized Linear Modeling
HR	Heart Rate
ICE	Intercurrent Events
ITT	Intent-to-treat
LAR	Legally Authorized Representative
LS	Least Squares
LVO	Large Vessel Occlusion
MAR	Missing at Random
MCMC	Markov Chain Monte Carlo
MedDRA	Medical Dictionary for Regulatory Activities
MI	Multiple Imputation
MNAR	Missing Not at Random
MRI	Magnetic Resonance Imaging
mRS	Modified Rankin Scale
n	Number of Observations
NCCT	Non-contrast Computed Tomography Scan
NIHSS	National Institutes of Health Stroke Scale
PD	Protocol Deviation
PP	Per Protocol
QTcF	QT interval corrected for heart rate using Fridericia's correction
RNA	Ribonucleic Acid
RSPL	Residual Pseudo-Likelihood with a Subject-specific expansion
SAE	Serious Adverse Event

Abbreviation	Definition
SAP	Statistical Analysis Plan
SDTM	Study Data Tabulation Model
TEAE	Treatment-emergent adverse event
TESAE	Treatment-emergent serious adverse event
TFL	Tables, Figures and Listings
TIA	Transient Ischemic Attack
VAS	Visual Analogue Scale
WHO	World Health Organization

1 INTRODUCTION

The purpose of this statistical analysis plan (SAP) is to provide a description of the statistical methods to be implemented for the analysis of data from the study with protocol number 06.5.1.H1 dated 09 May 2022 (version 2.4). The SAP will be finalized prior to database lock. Any future addendum to the SAP (if applicable) will be referenced in the Clinical Study Report (CSR).

The SAP will be considered as the governing document and in case of any differences between this SAP and the study protocol, the SAP will supersede the protocol.

2 STUDY OVERVIEW

2.1 Study Objectives

2.1.1 Primary Objective

The primary objective is to determine the safety of RNS60 in adult subjects (aged 18 years or older) with large vessel occlusion (LVO) and acute ischemic stroke (AIS) based on serious adverse events (SAEs) and 90-day mortality. To be eligible for this trial, subjects must have sudden onset of focal neurological symptoms within 24 hours prior to randomization consistent with an ischemic stroke. Symptoms must be persistent and present at the time of enrollment.

2.1.2 Secondary Objectives

The secondary objectives are to evaluate the efficacy of RNS60 in the following outcomes:

1. Reducing global disability (as measured using the Modified Rankin Scale [mRS] at 90 days)
2. Decrease in infarct volume (as defined by imaging at 48 hours)
3. Improving neurological outcome (as measured using the National Institutes of Health Stroke Scale [NIHSS] at 24 hours)
4. Reducing worsening of stroke, defined as progression, or hemorrhagic transformation, of the index stroke as documented by medical imaging that results in:

    (a) a life-threatening situation requiring intervention, and/or

    (b) increased disability as determined by a ≥4-point increase from lowest NIHSS pre decline, and/or

    (c) death by 48 hours or during hospital admission.
5. Reducing functional dependence (as defined by mean Barthel Index (BI) at Day 90)
6. Improving health-related quality of life, as measured by the EuroQol health-related quality of life (EQ-5D-5L) at Day 90

These outcomes will be assessed in the following hierarchical order (to control for the overall Type I error rate): first between high-dose RNS60 (1.0 mL/kg/h RNS60) compared to placebo, then both low and high dose RNS60 combined compared to placebo, and then between low-dose RNS60 (0.5 mL/kg/h RNS60) compared to placebo. In the analyses of efficacy, the combined-dose inference will be done within the statistical models by averaging the results in the two active doses and comparing to placebo. (This is in contrast to performing a separate

analysis in which subjects on active dose are treated as a single treatment arm.) The exception to this approach is the non-parametric analysis in Section 3.4.4.1, where the data in the two active arms are combined into a single group to be compared to placebo.

2.1.3 Attributes of the Estimands

The attributes of the main estimand (target of estimation), used for the primary analysis of the highest ranked efficacy endpoint (modified Rankin Scale (mRS) score) are defined below. Although the primary objective of this study is related to safety, analysis of the efficacy endpoints (secondary objectives of the study) will be based on a pre-defined hierarchy and therefore, an estimand is defined.

- The **population** is defined as the intent-to-treat study population (see Section 3.2.3).

- The corresponding **variable** to be obtained for each subject required to address the scientific question is disability based on the mRS score at Day 90. The mRS will be evaluated as a binary outcome (0-2, 3-6).

- The **population level summary** considered in this study is the odds ratio between RNS60 1.0 mL/kg/h and placebo 1.0 mL/kg/h.

- The **treatments** to be compared are RNS60 1.0 mL/kg/h and placebo 1.0 mL/kg/h. The treatments will be compared according to the randomized groups, regardless of the treatment that was actually received. In accordance with the treatment policy principle, all available data will be used in the analysis, regardless of e.g., premature discontinuation of study medication or administration of any concomitant treatment.

- The **intercurrent events** (ICEs) considered as potentially impacting results and/or interpretation are:
    - Premature discontinuation of study treatment
    - Death
    - Coronavirus Disease 2019 (COVID-19) pandemic related events (assessments that were missed due to COVID-19).

  For the data that are missing due to premature discontinuation of study treatment, an assumption of Missing At Random (MAR) will be made. Sensitivity analyses will be conducted using an assumption of Missing Not At Random (MNAR). No specific imputation will be done for these missing data, because the likelihood-based analysis approach can manage subjects with incomplete data under the assumption of MAR.

  For the data that are missing due to COVID-19, an assumption of MAR will be made for all analyses, both primary and sensitivity analyses.

  For the mRS, NIHSS and BI endpoints death is included as part of the scoring, so no imputation of these endpoints is required for subjects who die. Similarly, for the worsening of stroke endpoint, death represents a worsening. For the infarct progression end point, death will be represented by a missing value.

2.2 Study Design

2.2.1 Overview

This trial is a Phase 2, block urn randomized, blinded assessor, placebo-controlled, parallel group, two-dose (high and low), and longitudinal (pre-stroke, post-treatment, 48-hour, discharge, and Days 30 and 90) study with equal arm sample sizes (1:1:1). All participants in the trial will be followed for 90 days (or until death if prior to 90 days). Participants are considered to have completed the study if they have remained in the study until Day 90 or they have died prior to Day 90. The end of the trial is defined as the date the last enrolled participant has completed their Day 90 visit/contact.

The trial's primary aim is to demonstrate the safety of RNS60. As a secondary aim, the study will provide first data on whether RNS60 may reduce global disability, improve neurological outcome, reduce worsening of stroke and reduce infarction volumes. Superiority of the higher dose is hypothesized over placebo and of the lower dose over placebo is hypothesized for all secondary and tertiary objectives.

AIS large vessel occlusion participants who are selected for Endovascular Thrombectomy (EVT) will be given a 48-h infusion of either 0.5 mL/kg/h RNS60 (up to a maximum of 65 mL/h), 1.0 mL/kg/h RNS60 (up to a maximum of 130 mL/h), or 1.0 mL/kg/h (up to a maximum of 130 mL/h) placebo (normal saline) starting within 30 minutes of randomization (but prior to arterial access closure).

Randomization will be done with block urn randomization to balance age (<80 vs. ≥80), NIHSS score (6-10 vs. >10), and Alberta stroke program early computerized tomography score (ASPECTS) (5-7 vs. 8-10) for a total of 8 urns.

Table 1 shows the schedule of activities of randomized participants.

Table 1: Schedule of Activities for Randomized Participants

Visit/Contact	V1	V2	V3	V4	V5	V6/ End of Trial
Day	**Day 0 Baseline**	**Day 1 Post-EVT**	**Day 2/or early discharge**	**Day 6[1] or discharge**	**Day 30[2]**	**Day 90[2]**
Window		**(24 h+/-4h)**	**(48 h±4h)**		**(±5 d)**	**(-21 to +7d)**
Informed consent	X					
Regained capacity informed consent[3]			X	X	X	X
History and physical examination[4]	X	X	X			
Weight[5]	X		X			
Vital Signs (BP, HR, Temperature) [6]	X	X	X			
Chest radiograph	X[12]	X	X			
Electrocardiogram[7]	X[12]	X	X			
Randomization/ Study drug administration	X	X	X			
Continuous telemetry[7]	X	X	X			
Mortality		X	X	X	X	X
NIHSS[13]	X	X	X	X	X	X
mRS	X			X	X	X
Barthel Index	X				X	X
EQ-5D-5L						X
Imaging (NCCT & mCTA)	X					
Endovascular Procedure	X					
MRI head[8]		X	X			X
Laboratory Assessments	X[9]		X			
Pregnancy test[10]	X					
Blood collection for RNA analysis[11]		X	X			X
Blood collection for Plasma Biomarker		X	X			X
AE	Collected to Day 90					
SAE	Collected to Day 90					
Prior medications	X					
Concomitant medications	Collected to Day 90					

1. Visit will occur at Day 6 or hospital discharge if prior to Day 6.
2. At Day 30 and Day 90 it is preferred that participants will return to clinic. If an in-clinic visit is not possible the participant can be contacted by telemedicine (preferred) or by telephone (last option).
3. If the original process involved anyone other than the participant (and if required), site staff will make ongoing efforts until: (1) regained capacity consent is obtained from participant, (2) death, or (3) completion of the Day 90 assessment.
4. Physical exam every 8-12 hours during Day 1 and Day 2.
5. At baseline estimated or actual weight will be collected. If an estimated weight was collected at baseline, actual weight should be collected as soon as feasible and prior to discharge.
6. Vital signs (Blood Pressure (BP), Heart Rate (HR) only) will be recorded immediately before and after completion of the study drug infusion, and every 8-12 hours during Day 1 and Day 2, temperature will be collected at baseline only if standard of care.
7. Abnormal electrocardiogram or continuous telemetry result that suggests clinical instability will prompt a formal cardiology consult.
8. Magnetic Resonance Imaging (MRI) head with perfusion imaging within approximately 2h post-EVT on Day 1 and at 48h +/- 4h. MRI head only at 90d.
9. Blood should be drawn at baseline, but results are not required prior to randomization. Results from primary hospital (within 8 hours) are accepted.
10. If the participant is female and is of childbearing potential a pregnancy test (urine or serum point-of-care pregnancy test) must be collected at baseline, but result is not required prior to randomization.

11. Day 1 collection 12-24 h (+/-4 hours) after additional consent is obtained for optional collection.
12. Results from the baseline Electrocardiogram (ECG) and Chest X-ray(CXR) are not required prior to randomization.
13. NIHSS score is obtainable in-person or via telemedicine visit only (not over the phone).

D = days; h = hours

2.2.2 Randomization and Blinding

All subjects will be centrally assigned according to the study's Randomization and Study Product Management Plan to a randomized and blinded study intervention (i.e., RNS60 0.5 mL/kg/h, RNS60 1/0 mL/kg/h, or placebo 1.0 mL/kg/h) using block urn randomization tables (8 urns). The randomization table will be set for up to 126 subjects per urn to ensure no urn can be filled prior to enrollment completion. Each site will have its own randomization table. All subjects are identified by a 5-digit number (e.g., 01-001), consisting of a unique 2-digit site number followed by a 3-digit subject number. Each subject number is unique and will not be reused.

Treatment allocation will be assigned using 1:1:1 randomization of three arms with block urn randomization by age, NIHSS score, and ASPECTS. Randomized allocation will be fully concealed by having allocations based upon random assignment and blinded by the use of RNS60 and placebo infusion bags and labels with identical visual appearance. The lower rate of infusion associated with the low-dose RNS60 poses potential for unblinding of study staff involved with infusion administration to the low-dose group, however assessors and subjects will remain blinded. Each infusion bag will have a unique 5-digit identification number.

The purpose of using block urn randomization is to minimize the possibility of imbalance of treatment assignment by age, NIHSS score, and ASPECTS due to the small sample size. Block urn randomization provides consistent imbalance control but provides greater allocation randomness compared with permuted block design and can be used for more than three arms.

All participants, investigators, their clinical staff, local laboratories, study personnel, the data management group, and the sponsor staff and delegates will be blinded to the randomization codes and study drug assignment. All study personnel (Contract Research Organization (CRO) and Sponsor and sites) will remain blinded to this data until database lock, at minimum. In the event of emergency unblinding due to participant safety, the Investigator may request unblinding of that participant only. Processes and procedures for unblinding will be accordance with the study protocol, Data Management Plan, Safety Management Plan, and any applicable CRO or Sponsor Standard Operating Procedures.

2.2.3 Study Drug

RNS60 is [redacted] RNS60 has demonstrated exceptional safety in rodent and non-human primate toxicity studies. Additionally, RNS60 has demonstrated significant efficacy in a non-human primate model of acute ischemic stroke without any indications of adverse effects. In all clinical studies to date, RNS60 has been well tolerated. In this study, infusion will begin prior to the completion of the endovascular revascularization procedure (defined as the time of arterial access closure) and complete 48h after the infusion is initiated.

2.2.4 Sample Size Determination

As the primary aim of this proof-of-concept study is to demonstrate safety, no power analysis was conducted for sample size determination. The trial targets to enroll a total number of 100 participants aged 18 years or older with LVO AIS and who are selected for endovascular revascularization.

Anticipated attrition factors include death from stroke unrelated to treatment (10%), discontinuation of treatment due to symptoms of fluid overload (20%), and early discharge (5%).

2.3 Study Endpoints

2.3.1 Primary Safety Endpoints

The primary safety endpoints include:

1) Frequencies and severities of SAEs (see Appendix C for definition of SAE).
2) 90-day mortality (as a proportion).

2.3.2 Secondary Efficacy Endpoints

Secondary endpoints include:

1) Disability based on the mRS score at Day 90 as a binary outcome (0-2, 3-6).

   The mRS is a commonly used valid and reliable clinician-reported outcome measure in subjects who have suffered a stroke. It measures functional recovery (the degree of disability or dependence in daily activities in a 6-point disability scale with possible scores ranging from 0 to 5. A score of 6 is used for subjects who expire.

   Table 2. Description of scores in mRS

Score	Description
0	No symptoms at all
1	No significant disability despite symptoms; able to carry out all usual duties and activities
2	Slight disability: unable to carry out all previous activities but able to look after own affairs without assistance
3	Moderate disability: requiring some help, but able to walk without assistance
4	Moderately severe disability: unable to walk without assistance and unable to attend to own bodily needs without assistance
5	Severe disability; bedridden, incontinent, requiring constant nursing care and attention
6	Death (at hospital or after discharge)

   The post-dose mRS score will be obtained at Day 6 (or discharge), and Days 30 and 90. Premorbid mRS status may be obtained at any time, but ideally at the Day 1 or 2 visit. The mRS will only be scored by those trained and certified in the use of this scale.

2) Infarct volume of stroke at 48h.

   Infarct progression/regression as measured by MRI of the brain in RNS60 versus placebo control participants will be calculated from the 48h imaging and compared to the

immediate post-thrombectomy MRI. The volume of injured tissue on Fluid Attenuated Inversion Recovery (FLAIR) and T2 weighted images will be compared.

3) NIHSS score at hour 24.

The NIHSS is a standardized neurological examination scale that is a valid and reliable measure of disability and recovery after acute stroke. The NIHSS assessment is a standardized 15-item impairment scale, intended to evaluate neurologic outcome and degrees of recovery for subjects with stroke. The scale assesses levels of consciousness, extraocular movements, visual fields, facial muscle function, extremity strength, sensory function, coordination (ataxia), language (aphasia), speech (dysarthria), and hemi-inattention (neglect). The NIHSS will be administered at baseline, post-EVT (2h), 24h, 48h, Day 6 (or discharge), Day 30 and Day 90. The NIHSS will only be scored by those trained in the use of this scale.

Table 3. Scales and score range in NIHSS

Item	Scales	Score range
1a	Level of Consciousness	0-3
1b	LOC Questions	0-2
1c	LOC Commands	0-2
2	Best Gaze	0-2
3	Visual	0-3
4	Facial Palsy	0-3
5a	Motor Arm –Left Arm	0-4
5b	Motor Arm –Right Arm	0-4
6a	Motor Leg –Left Leg	0-4
6b	Motor Leg – Right Leg	0-4
7	Limb Ataxia	0-2
8	Sensory	0-2
9	Best Language	0-3
10	Dysarthria	0-2
11	Extinction and Inattention	0-2

Scoring:

Each item is scored in the respective ranges listed in Table 3 (0-2, 0-3, or 0-4), and untestable items are scored as “UN”. A score of 0 indicates normal performance. Total scores on the NIHSS range from 0-42, with higher values reflecting increasing severity.

Stroke severity is further stratified in the following way (Brott, Adams, Olinger, Marler, Barsan, Biller, & Hertzberg, 1989):

> 25: Very severe

15-24: Severe

5-14: Mild to moderately severe

< 5: Mild

The predictive value of the scale can also aid in planning a subject’s rehabilitation or long-term care needs as early as on the day of admission. NIHSS scores can be

interpreted in the following way (Schlegel, D., Kolb, S. J., Luciano, J. M., Tovar, J. M., Cucchiara, B. L., Liebeskind, D. S., & Kasner S. E., 2003):

≥ 14 – Severe: Long-term care in nursing facility required

6-13 – adequate: Acute inpatient rehabilitation required

≤ 5 – Mild: 80% with this score are discharged home

4) The proportion of study subjects with worsening of stroke at the 90-day study period.

Worsening of stroke is defined as progression, or hemorrhagic transformation of the index stroke, as documented by brain imaging, which is (a) life-threatening requiring intervention and/or (b) results in increased disability as gauged by a ≥ 4-point increase from lowest National Institutes of Health Stroke Scale (NIHSS) pre decline and/or (c) results in death.

5) Barthel Index at Day 90.

The BI is an index of functional independence. Its values range from 0 to100, with higher scores indicating greater independence.

Table 4. Scales and score range in BI

Item	Score range
Feeding	0-10
Bathing	0-5
Grooming	0-5
Dressing	0-10
Bowels	0-10
Bladder	0-10
Toilet use	0-10
Transfers (bed to chair and back)	0-15
Mobility (on level surfaces)	0-15
Stairs	0-10

Item scores vary in increments of 5 points.

6) Health-related quality of life, as measured by the EuroQol health-related quality of life (EQ-5D-5L) at Day 90.

EQ-5D-5L is developed by EuroQol Research Foundation and is a generic instrument for measuring and describing health-related quality of life.

It consists of two elements, a 5-item questionnaire and an EQ-Visual Analogue Scale (VAS). Both elements are interviewer administered by study staff.

EQ-5D-5L is a version that further improves the sensitivity of EQ-5D-3L, provides respondents with a wider range of options to describe their health and is comprised of five response levels to five different dimensions.

- 5-Item Questionnaire: The questionnaire provides a simple descriptive profile of health state. The questionnaire comprises 5 dimensions (mobility, self-care, usual activities, pain/discomfort, anxiety/depression) and each dimension has 5 response levels (1-no problems, 2-slight problems, 3-moderate problems, 4-severe problems,

5-unable to/extreme problems). The resulting 5-digit code corresponds to the response of each dimension of the health state. For instance, 11111 and 55555 indicate the best health condition and the worst health condition, respectively.

- Index Score: The health state is summarized to be a single number, EQ-5D-5L Index score, by applying standard value set. A representative sample of the general population in a country/region is asked to place a value on health states and the standard value set is developed. The standard value set varies by country and reflects what health state is regarded as good according to country-specific preferences. Refer to the paper written by Pickard and his colleagues (2019) for the U.S. standard value set.

EQ-Visual Analogue Scale (VAS): EQ-VAS provides an alternative way to measure subjects' perception about their overall current health. The subject is asked to mark X on the scale to indicate how their health is 'TODAY'. This scale consists of numbers from 0 (the worst health you can imagine) to 100 (the best health).

3 STATISTICAL METHODOLOGY

3.1 General Considerations

3.1.1 Analysis Day

Analysis days will be calculated from the date of starting infusion of study drug. The day on which infusion of study drug is started is Day 1, and the day immediately before Day 1 is Day -1. For the purpose of this statistical analysis there is no Day 0.

3.1.2 Analysis Visits

Scheduled visits will be assigned to analysis visits as recorded on the case report form (CRF). Unscheduled and early termination visits will be assigned to analysis visits according to the analysis windows in Table 5. The record occurring on the target day will be picked for the time point analysis. If the record on a target day does not exist, then the one closest to the target date will be used.

Table 5. Analysis windows

Analysis Visit	Target Analysis Day	Low Analysis Day	High Analysis Day
Baseline	-1		-1
DAY 1	1	1	1
DAY 2	2	2	3
DAY 6	6	4	8
DAY 30	30	9	44
DAY 90	90	45	104

3.1.3 Definition of Baseline

Baseline is defined as the last measurement prior to start of the study drug infusion. Baseline blood work, ECG and CXR (Day 1) are not required prior to randomization or start of study drug infusion. Baseline infarct volume indicates the initial infarct volume as obtained from the first MRI approximately 2 hours post-endovascular thrombectomy on Day 1.

3.1.4 Summary Statistics

Categorical data will generally be summarized with counts and percentages of subjects. The denominator used for the percentage calculation will be clearly defined. Continuous data will generally be summarized with descriptive statistics including n (number of non-missing values), mean, median, standard deviation, interquartile range (Q1, Q3), minimum, and maximum.

3.1.5 Evaluation of Site Effect

Site will be modeled as a random effect to account for variability in the outcome due to the differences between sites. A subgroup analysis for each site will be run.

3.1.6 Handling of Dropouts and Missing Data

For participants who have missing observations on 90-day mRS, 24-hour NIHSS, worsening stroke, or infarct volume, the missing data in the primary analyses will be managed with an assumption of MAR using longitudinal mixed models. In addition, every effort will be made to complete assessments using the participants legally authorized representative (LAR) for information if needed. Assessments with missing elements will be considered missing as a whole. Missing dates will be queried at the site level prior to database lock.

3.1.7 Handling of Death

Subjects who die during the study will be scored as follows for all scheduled visits following the death: 6 for mRS, 42 for NIHSS and 0 for BI. The value 6 for mRS is part of the mRS definition. The values for NIHSS and BI are conventions, consistent with the worst possible score.

3.2 Analysis Populations

3.2.1 Enrolled Population

The enrolled population is defined as all subjects randomized into the trial.

3.2.2 Randomly Assigned to Study Intervention Population

The randomly assigned to study intervention population is defined as all subjects who received a dose or partial dose of study drug.

3.2.3 Intent-to-Treat (ITT) Population

The ITT Population will be the primary population for analysis of efficacy data.

The ITT Population is defined as all randomized subjects who received study drug. Their data will be analyzed according to the randomized treatment, regardless of treatment actually received. Subjects who receive treatment but do not get the full 48 hours of treatment will be included in the ITT analysis. The full 48-hour treatment time is considered +/- 4 hours from the intended infusion stop time. Any randomized subjects who did not receive treatment will be excluded from the ITT population.

3.2.4 Per-Protocol (PP) Population

The PP Population is defined as all subjects who are randomized and receive study drug with no major protocol deviations (PD) that may impact the efficacy assessments. A major PD that would preclude a subject from being included in the PP Population could involve, but is not limited to the following:

- Inclusion/exclusion criteria not met
- Receiving treatment without consent
- Receiving incorrect dose volume (outside of the 48-hour infusion (+/-4 hours)
- Receiving the wrong study drug/placebo IV bag

The PP Population is a secondary population for analysis of the efficacy data. The primary analysis will be repeated on the PP Population. The final criteria for reasons to exclude subjects from the PP Population, along with a list of subjects with major PDs leading to exclusion from the PP Population will be finalized prior to unblinding the randomized treatment assignments.

3.2.5 Safety Population

The Safety Population is defined as all randomized subjects who receive any volume of study drug. All safety data will be analyzed using the Safety Population. In the event that a subject is given the incorrect study drug (i.e., received the incorrect assigned randomized study drug), the actual treatment received will be used for analysis.

3.3 Subject Data and Study Conduct

3.3.1 Subject Disposition

Subject disposition will be listed by arm.

The number and percentage of subjects in each of the following categories will be presented by treatment and in total for all enrolled subjects:

- Enrolled Population,
- Randomly assigned to study intervention Population,
- ITT Population,
- PP Population,
- Safety Population,
- Completed the study,
- Early terminated the study and if the primary reason is due to COVID-19.

Primary reasons for discontinued study and primary reasons for discontinued study due to COVID-19 will be tabulated.

3.3.2 Protocol Deviations

A CSR reportable PD is related to inclusion/exclusion criteria, conduct of the trial, subject management or subject assessment that impact the safety of the subjects or jeopardize the quality of study data. Counts and percentages of subjects with PDs by deviation category, as defined in the protocol deviation plan, will be summarized by treatment and in total based on the Enrolled Population.

Primary reasons for excluding a subject from the PP Population will be summarized.

The following categories will be used for CSR reportable PDs:

- A subject did not meet entry criteria

- A subject received treatment without consent
- A subject was not approached for consent despite regaining capacity
- A subject received the wrong study drug/placebo IV bag
- A subject received an incorrect dose and/or infusion rate
- A subject started study drug/placebo dosing after the mechanical endovascular thrombectomy was completed (arterial access closure)
- Safety labs or assessments were not completed as defined by protocol
- An SAE which was not reported within 24 hours after event was known

Protocol deviations determined to have been related to COVID-19 will be listed. A listing of COVID-19 related non-reportable PDs will be included in an appendix of the CSR.

3.3.3 Demographic and Baseline Characteristics

The following demographic and baseline characteristics will be summarized:

- Age (years) and age categories (<80 years, ≥80 years)
- Sex
- Childbearing potential
- Race
- Ethnicity
- Weight (kg)
- NIHSS and NIHSS categories at baseline (6-10, >10)
- ASPECTS and ASPECTS categories (5-7, 8-10)

Demographic and baseline characteristics will be summarized with descriptive statistics or counts and percentages of subjects as appropriate by treatment and in total for each defined analysis population.

3.3.4 Medical History

Medical history will be coded to system organ class and preferred term using the medical dictionary for regulatory activities (MedDRA) version 24.0. Counts and percentages of subjects with medical history by system organ class and preferred term will be summarized by treatment and in total based on the Enrolled Population. A medical review of the coding reports will be performed prior to database lock.

Stroke details such as stroke mechanism and symptomatic intracranial occlusion locations will also be summarized.

Comorbidities (such as autoimmune disease, cancer, active infection, COVID-19, hypertension, Transient Ischemic Attack (TIA), Cardiovascular accident (CVA), intercranial hemorrhage, head or spine trauma within past 3 months), smoking status, and additional medical history such as concomitant diseases or past surgeries will be listed.

3.3.5 Concomitant Medications

Any medication or vaccine (including over the counter or prescription medicines) that the participant is receiving at the time of enrollment through the Day 90 visit will be recorded. There are no restricted medications in this study. Concomitant medications will be coded to anatomical

therapeutic chemical (ATC) class and preferred term using the world health organization (WHO) Drug Dictionary B3 Global, March 2021. A medical review of the coding reports will be performed prior to database lock.

For summary purposes, medications will be considered prior medications if they were started prior to the date of starting infusion of study drug and not administered during infusion and concomitant medications if they were taken before and were continuing after the date of starting infusion of study drug or initiated after the date of starting infusion of study drug.

If a medication has incomplete start or stop dates, dates will be imputed to determine whether a medication should be considered prior or concomitant. If a medication start date is incomplete, the first day of the month will be imputed for missing day and January will be imputed for missing month. If a medication stop date is incomplete, the last day of the month will be imputed for missing day and December will be imputed for missing month. Incomplete start and stop dates will be listed as collected without imputation.

Counts and percentages of subjects taking prior and concomitant medications by ATC class and preferred term will be summarized by treatment and in total based on the Safety Population.

In addition, counts and percentages of COVID-19 vaccination, medications taken during the time of infusion, medications taken for an Adverse Event (AE), and any medications that the participant is receiving at the time of enrollment up to Day 90, including the type of intravenous thrombolysis tPA/TNK will also be summarized by treatment and in total based on the Safety Population.

3.3.6 Eligibility Criteria

Unmet eligibility criteria (inclusion not met/exclusion criteria met), date and time of stroke symptom onset (or if not known, last known well), whether the stroke symptom(s) was witnessed, whether subject experienced stroke on awakening, dates and times of informed consent signed, how consent was obtained (use of LAR) and randomization (study drug assignment) will be listed for the ITT Population.

3.3.7 Study Drug Exposure and Compliance

Total time of exposure to study drug will be calculated as stop time of infusion minus start time of infusion in units of hours (to the closest tenth). Note that the exposure calculation is intended to describe the length of time a subject was exposed to study drug and therefore does not take study drug interruptions into account. Time of exposure to study drug will be summarized by treatment assignment and in total based on the Safety Population with descriptive statistics. Infusion interruption is defined as study drug infusion stopped > 60 minutes.

Percent compliance (per subject) to the study drug regimen will be calculated as:

> 100 x total time of infusion / 48 hours.

Total time of infusion (per subject) will be calculated as:

> (stop time of infusion – start time of infusion) – (restart time of infusion – suspend time of infusion).

If the infusion is not interrupted, the stop time (restart time of infusion – suspend time of infusion) will be considered 0 for the compliance calculation. Percent compliance to the study drug regimen will be summarized by treatment and in total based on the Safety Population with

descriptive statistics and with counts and percentages of subjects with compliance in the following categories:

- <80%
- 80-120%
- >120%

3.4 Safety and Efficacy Assessments

The primary safety analysis will be conducted in the Safety Population. The secondary efficacy analysis will be conducted in the ITT and PP Populations.

3.4.1 Primary Safety Endpoints

Frequencies and severities of SAEs

The proportion of participants with SAEs through Day 90 between the three treatment groups (RNS60 0.5 mL/kg/h, RNS60 1.0 mL/kg/h, and placebo 1.0 mL/kg/h)

In addition, the frequency and severity categories will be summarized with counts and percentages of SAEs by treatment and site and in total. See Appendix C for definition of SAE.

90-day Mortality

The proportion of participants alive at Day 90 between the three treatment groups (RNS60 0.5 mL/kg/h, RNS60 1.0 mL/kg/h, and placebo 1.0 mL/kg/h).

3.4.2 Secondary Efficacy Endpoints

All outcomes will be assessed in the following hierarchical order (to control of the overall Type I error rate). As it is hypothesized that higher dosage will result in less disability, subjects receiving placebo 1.0 mL/kg/h will first be compared to RNS60 1.0 mL/kg/h and then, as a lower ranking secondary endpoint, combined RNS60 doses (1.0 mL/kg/h and 0.5 mL/kg/h) compared to placebo, and finally RNS60 0.5 mL/kg/h compared to placebo. Specifically, efficacy of each of the 5 key endpoints will be assessed between high-dose RNS60 compared to placebo, followed by the same endpoints between combined RNS60 doses (1.0 mL/kg/h and 0.5 mL/kg/h) compared to placebo, finally followed by the same 5 endpoints between RNS60 low dose (0.5 mL/kg/h) compared to placebo:

1. Disability based on mRS score, high-dose vs placebo
2. Infarct progression/regression, high-dose vs placebo
3. NIHSS score, high-dose vs placebo
4. Worsening of stroke high-dose vs placebo
5. BI, high-dose vs placebo
6. Disability based on mRS score, combined-dose vs placebo
7. Infarct progression/regression, combined-dose vs placebo
8. NIHSS score, combined-dose vs placebo
9. Worsening of stroke, combined-dose vs placebo

10. BI, combined-dose vs placebo
11. Disability based on mRS score, low-dose vs placebo
12. Infarct progression/regression, low-dose vs placebo.
13. NIHSS score, low-dose vs placebo
14. Worsening of stroke, low-dose vs placebo
15. BI, low-dose vs placebo

If a comparison listed above is determined to be not statistically significant, then the remaining comparisons in the hierarchy will be tested in any case and interpreted in an exploratory manner.

In addition to the primary time point of each endpoint (as defined below), all other applicable time points will be compared as well and interpreted in an exploratory manner.

In these analyses, testing the combined-dose versus placebo will be done within the statistical models by averaging the results in the two active doses and comparing to placebo. (This is in contrast to performing a separate analysis in which subjects on active dose are treated as a single treatment arm.)

Generalized linear mixed models will be implemented for the analyses of disability based on mRS, infarct volume, NIHSS at 24 hours, worsening of stroke, and Barthel Index. NIHSS will be analyzed on the recorded scale of measurements and the treatment groups' Least Squares (LS) means, corresponding 95% confidence intervals (CIs), and LS mean differences will be reported. Disability based on mRS, worsening of stroke, and Barthel Index are dichotomized endpoints that will be analyzed on the logit scale of measurement. The resulting LS means and confidence intervals will be back-transformed to probabilities, and differences in least squares means will be reported as odds ratios. Infarct volume will be analyzed as log-normally distributed, and descriptive statistics will be geometric means where between-group comparisons will be reported as ratios of geometric means. Covariates baseline NIHSS and ASPECTS binary factors for efficacy analysis will use baseline values from the Elecronic Data Caputre.

1. Modified Rankin Scale (mRS) score:

The effect of treatment on global disability at Day 90 will be examined by comparing the proportion of subjects who had disability (Yes or No) between the three treatments (RNS60 1.0 mL/kg/h, RNS60 0.5 mL/kg/h, and placebo 1.0 mL/kg/h) using generalized linear mixed modeling (GLM) using residual pseudo-likelihood with a subject-specific expansion(RSPL) assuming a binary outcome (0-2=0, 3-6=1). Covariates include the Age, baseline NIHSS, and ASPECTS binary factors used for block urn randomization and baseline (pre-morbid) mRS, baseline occlusion location, study site, baseline perfusion status, and log of baseline infarct volume. Treatment, visit day, and interaction of treatment and visit day are included as categorical fixed effects.

An unstructured covariance structure will be applied for GLM. In case the model will not converge with the unstructured covariance structure, Heterogeneous Autoregressive (1) (ARH(1)), Heterogeneous Compound Symmetry (CSH), Autoregressive(1) (AR(1)) or Compound Symmetry (CS) covariance structure will be used instead in this order until convergence is achieved.

The predicted probability for each treatment group and least squares (LS) mean difference between high-dose and placebo and low-dose and placebo groups along with the 95% confidence intervals (CIs) for Day 90 will be provided using the LSMEANS/DIFF statement with the ILINK option. The comparison of the two combined active arms versus placebo will be performed using the LSMESTIMATE statement. The odds ratio will also be calculated. The P-value for the hypothesis testing will also be provided. Treatment difference will be assessed with a 2-sided alpha level of 0.05, verifying, however that any statistically significant finding is consistent with the alternative hypothesis of efficacy versus placebo.

The sample SAS code planned for the analysis is outlined below.

Summary of dichotomized mRS endpoint (0-2 indicating a value of 0 and 3-6 indicating a value of 1) with counts and percentages of subjects will be generated to compare baseline mRS with Day 90 for each treatment arm and for the two active arms combined.

Horizontal bar graphs will be generated to visualize mRS distribution at Day 90 across all treatment groups. mRS score at Day 90 will be displayed where bars will be labelled with subject counts and proportions.

KEY SENSITIVITY ANALYSIS (analysis assuming Missing Not At Random (MNAR))

The analysis consists of the following steps:

1. Imputation of all intermittent missing data with multiple imputation (MI) assuming MAR, in order to generate a dataset with monotone missing data structure. The endpoint being modeled will be the mRS on the original 0-6 scale, rather than the derived binary endpoint.
2. Based on dataset generated in Step 1: imputation of all monotone missing data with MI assuming MAR, in order to generate dataset with no missing data
3. Based on dataset generated in Step 2 (with no missing data): all visits that have monotone missing data that are not due to COVID-19 will be set back as missing for subjects in the active arm.
4. Based on dataset generated in Step 3: imputation of remaining monotone missing data (not related to COVID-19) with multiple imputation assuming MNAR (Copy-Reference imputation).
5. Based on data generated in step 4, analysis of imputed data with GLMs
6. Combination of results from GLMs.

Step 1: Imputation of intermittent missing data

Based on dataset generated in Step 1, a seed value of 2001 will be used. The imputation is based on the MAR assumption, i.e. the missing data are assumed to follow the same model as for the other subjects in their respective treatment arm. Age, baseline NIHSS, and baseline ASPECTS are included as a factor in the model because the randomization is stratified by these factors.

The following SAS code will be used to generate the monotone missing data pattern:

If any of the treatment levels has no missing data, the data from the treatment level in question will be excluded from the PROC MI procedure above (using WHERE statement). If this is done, the observed data from the treatment level in question (without any missing data) will be added to the output dataset produced above 500 times, i.e. once for each round of imputation.

Step 2: Imputation of missing data with assumption of MAR

Using the dataset generated in Step 1, all remaining missing values will be imputed based on the assumption of MAR. The following SAS code will be used for the imputation assuming MAR.

[redacted]

Step 3: Setting monotone missing data that are not due to COVID-19 back as missing

After Step 2, information on reason for missing data (by subject and visit) will be merged to the dataset that contains no missing data. Within each subject, data from the visits that have monotone missing data that are not due to COVID-19 will be set back as missing for subjects in the active arms.

Step 4: Imputation of remaining missing data (non COVID-19 related) with assumption of MNAR

The dataset generated in Step 3 (which contains monotone missing data) will be imputed based on the assumption of MNAR, using Copy-Reference imputation. The MNAR imputation will be based on trajectories in the placebo group, i.e., data from the placebo group only will be used to generate the imputations. This step will include data for active subjects identified in step 3 along with all placebo subjects. For this, the MNAR statement will be used as described below.

[redacted]

Step 5: Analysis of imputed data with GLM

The imputed datasets generated in Step 4 do contain only non-missing values and are used as input in the statistical model. The final step before statistical inference is to convert the imputed mRS data (on the 0-6) scale to the binary endpoint (0-2, 3-6). Residual pseudo-likelihood with a subject-specific expansion (RSPL) based GLMs will be run on each of the 500 generated imputed datasets. For additional details on how to specify the GLM, see the analysis section corresponding with this sensitivity analysis.

Step 6: Combination of results from the GLMs

Finally, the MIANALYZE procedure in SAS will be applied to combine the 500 results from the analyses generated in Step 4, to derive an estimate of the treatment difference at day 90. In addition to the estimate, corresponding two-sided 95% CIs and p-values will be generated with the MIANALYZE procedure.

2. Volume of stroke or Infarct volume (Diffusion-weighted imaging, DWI):

Infarct progression/regression will be analyzed as measured by MRI brain imaging. Where MRI was not available, infarct volumes will be obtained from a 48-hour CT imaging. Infarct volume will

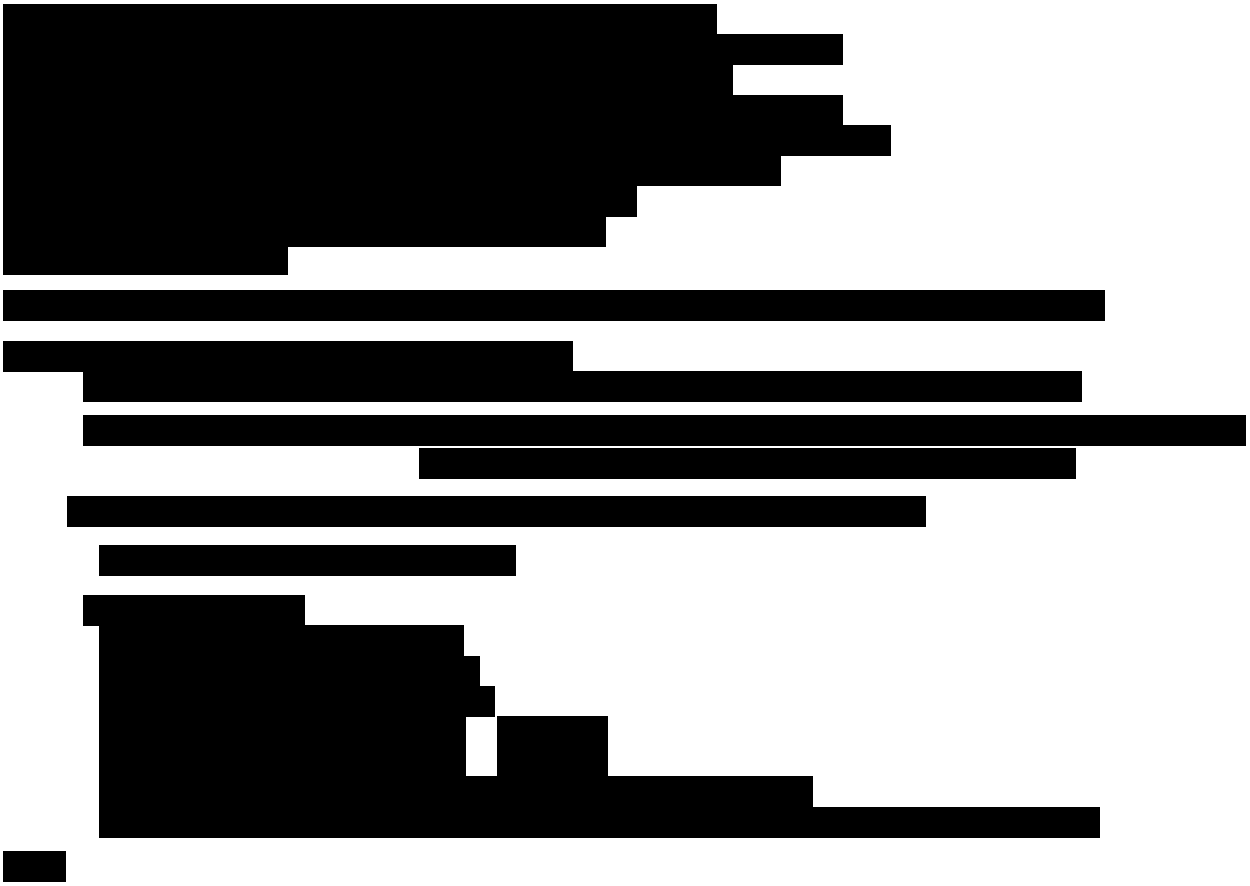
be summarized with descriptive statistics at Baseline, 24 hour, 48 hour, and Day 90 timepoints. The volume difference between 48 hour and 24 hour and between Day 90 and 24 hour timepoint will also be summarized.

The simple effect of treatment on Infarct progression/regression will be examined by 48-hour infract volume on a log scale between the three treatments (RNS60 1.0 mL/kg/h, RNS60 0.5 mL/kg/h, and placebo 1.0 mL/kg/h) using GLM using maximum likelihood assuming a Gaussian distribution. Covariates include the log of baseline infarct volume along with Age, baseline NIHSS, ASPECTS binary factors used for block urn randomization, baseline perfusion status, baseline occlusion location, and study site.

The geometric mean for each treatment group and ratios of geometric means based on LS mean differences between full-dose and placebo and low-dose and placebo groups along with the 95% CIs will be provided using an the LSMESTIMATE statement with the ILINK option. The comparison of the two combined active arms versus placebo will also be performed using an LSMESTIMATE statement. P-values for the hypothesis testing will also be provided. Treatment difference will be assessed with a 2-sided alpha level of 0.05.

The sample SAS code planned for the analysis is outlined below.

****



KEY SENSITIVITY ANALYSIS (analysis assuming MNAR)

The analysis consists of the following steps:

1. Imputation of all intermittent missing data with multiple imputation assuming MAR, in order to generate a dataset with monotone missing data structure
2. Based on dataset generated in Step 1: imputation of all monotone missing data with multiple imputation assuming MAR, in order to generate dataset with no missing data
3. Based on dataset generated in Step 2 (with no missing data): all visits that have monotone missing data that are not due to COVID-19 will be set back as missing for subjects in the active arms
4. Based on dataset generated in Step 3: imputation of remaining monotone missing data (not related to COVID-19) with multiple imputation assuming MNAR (Copy-Reference imputation)
5. Based on dataset generated in Step 4, analysis of imputed data with GLMs
6. Combination of results from the GLMs.

Step 1: Imputation of intermittent missing data

Multiple imputation techniques will be applied in the ITT analysis set. For subjects with intermittent missing values, a monotone missingness pattern will be generated. Intermittent missing values will be imputed using the MCMC methodology which assumes a multivariate normal distribution over all variables included in the imputation model. The MI procedure in SAS will be used for this purpose and this first MI step will be repeated 500 times, generating 500 different datasets. A random seed value will be used in the MI procedure and documented in this SAP, to allow replication of the analysis. Seed value of 2002 will be used. The imputation is based on the MAR assumption, i.e., the missing data are assumed to follow the same model as the other subjects in their respective treatment arm. Age, baseline NIHSS, and baseline ASPECTS are included as a factor in the model because the randomization is stratified by these factors. Baseline infarct volume and 48 hour infarct volume will be log transformed prior to imputation. The following SAS code will be used to generate the missing data pattern:

If any of the treatment levels has no missing data, the data from the treatment level in question will be excluded from the PROC MI procedure above (using WHERE statement). If this is done, the observed data from the treatment level in question (without any missing data) will be added to the output dataset produced above 500 times, i.e. once for each round of imputation.

Step 2: Imputation of missing data with assumption of MAR

Using the dataset generated in Step 1, all remaining missing values will be imputed based on the assumption of MAR. The following SAS code will be used for the imputation assuming MAR.

Step 3: Setting monotone missing data that are not due to COVID-19 back as missing

After Step 2, information on reason for missing data (by subject and visit) will be merged to the dataset that contains no missing data. Within each subject, data from the visits that have monotone missing data that are not due to COVID-19 will be set back as missing for subjects in the active arms.

Step 4: Imputation of missing data with assumption of MNAR

The dataset generated in Step 3 will be imputed based on the assumption of MNAR, using Copy-Reference imputation. The MNAR imputation will be based on the placebo group, i.e. data from the placebo group only will be used to generate the imputations. For this, the MNAR statement will be used as described below.

[redacted]

Step 5: Analysis of imputed data with GLM

The imputed datasets generated in Step 4 do contain only non-missing values and are used as input in the statistical model. RSPL based GLMs will be run on each of the 500 generated imputed datasets. For additional details on how to specify the GLM, see the analysis section corresponding with this sensitivity analysis.

Step 6: Combination of results from the GLMs

Finally, the MIANALYZE procedure in SAS will be applied to combine the 500 results from the analyses generated in Step 3, to derive an estimate of the treatment difference at hour 48. In addition to the estimate, corresponding two-sided 95% CIs and p-values will be generated with the MIANALYZE procedure.

3. NIHSS score:

The simple effect of treatment on neurological functioning at 24 hours will be examined by 24-hour NIHSS scores between the three treatments (RNS60 1.0 mL/kg/h, RNS60 0.5 mL/kg/h, and placebo 1.0 mL/kg/h) using generalized linear modeling using maximum likelihood assuming a normal distribution. Covariates include the Age and baseline ASPECTS binary factors used for block urn randomization along with baseline NIHSS, log of baseline infarct volume, baseline perfusion status, baseline occlusion location, and study site. Spaghetti plots displaying NIHSS scores for each subject over time will also be generated.

The LS means for each treatment group and LS mean difference between high-dose and placebo and low-dose and placebo groups along with the 95% CIs will be provided using the LSMEANS/DIFF statement. The comparison of the two combined active arms versus placebo will be performed using the LSMESTIMATE statement. The P-value for the hypothesis testing will also be provided. Treatment difference will be assessed with a 2-sided alpha level of 0.05.

The sample SAS code planned for the analysis is outlined below.

KEY SENSITIVITY ANALYSIS (analysis assuming MNAR)

The analysis consists of the following steps:

1. Imputation of all intermittent missing data with MI assuming MAR, in order to generate a dataset with monotone missing data structure.
2. Based on dataset generated in Step 1: imputation of all monotone missing data with MI assuming MAR, in order to generate dataset with no missing data
3. Based on dataset generated in Step 2 (with no missing data): all visits that have monotone missing data that are not due to COVID-19 will be set back as missing for subjects in the active arms
4. Based on dataset generated in Step 3: imputation of remaining monotone missing data (not related to COVID-19) with multiple imputation assuming MNAR (Copy-Reference imputation)
5. Based on dataset generated in Step 4, analysis of imputed data with GLMs
6. Combination of results from the GLMs.

Step 1: Imputation of intermittent missing data

Multiple imputation techniques will be applied in the ITT analysis set. For subjects with intermittent missing values, a monotone missingness pattern will be generated. Intermittent missing values will be imputed using the Markov Chain Monte Carlo (MCMC) methodology which assumes a multivariate normal distribution over all variables included in the imputation model. The MI

procedure in SAS will be used for this purpose and this first MI step will be repeated 500 times, generating 500 different datasets. A random seed value will be used in the MI procedure and documented in this SAP, to allow replication of the analysis. Seed value of 2003 will be used. The imputation is based on the MAR assumption, i.e., the missing data are assumed to follow the same model as the other subjects in their respective treatment arm. Age, NIHSS, and ASPECTS are included as a factor in the model because the randomization is stratified by these factors. The following SAS code will be used to generate the missing data pattern:

[redacted]

If any of the treatment levels has no missing data, the data from the treatment level in question will be excluded from the PROC MI procedure above (using WHERE statement). If this is done, the observed data from the treatment level in question (without any missing data) will be added to the output dataset produced above 500 times, i.e. once for each round of imputation.

Step 2: Imputation of missing data with assumption of MAR

Using the dataset generated in Step 1, all remaining missing data will be imputed based on the assumption of MAR. The following SAS code will be used for the imputation assuming MAR.

[redacted]

Step 3: Setting monotone missing data that are not due to COVID-19 back as missing

After Step 2, information on reason for missing data (by subject and visit) will be merged to the dataset that contains no missing data. Within each subject, data from the visits that have monotone missing data that are not due to COVID-19 will be set back as missing for subjects in the active arms.

Step 4: Imputation of missing data (non COVID-19 related) with assumption of MNAR

The dataset generated in Step 3 will be imputed based on the assumption of MNAR, using Copy-Reference imputation. The MNAR imputation will be based on the placebo group, i.e., data from the placebo group only will be used to generate the imputations. For this, the MNAR statement will be used as described below.

[redacted]

Step 5: Analysis of imputed data with GLM

The imputed datasets generated in Step 4 do contain only non-missing values and are used as input in the statistical model. RSPL based GLMs will be run on each of the 500 generated imputed datasets. For additional details on how to specify the GLM, see the analysis section corresponding with this sensitivity analysis.

Step 6: Combination of results from the GLMs

Finally, the MIANALYZE procedure in SAS will be applied to combine the 500 results from the analyses generated in Step 3, to derive an estimate of the treatment difference at hour 24. In addition to the estimate, corresponding two-sided 95% CIs and p-values will be generated with the MIANALYZE procedure.

Additionally, neurological functioning at Day 90 will be examined by NIHSS scores at Day 90 between the three treatments (RNS60 1.0 mL/kg/h, RNS60 0.5 mL/kg/h, and placebo 1.0 mL/kg/h). GLM analysis and missing data imputation analysis at Day 90 will be performed using the same approach described above for neurological functioning using 24-hour NIHSS scores.

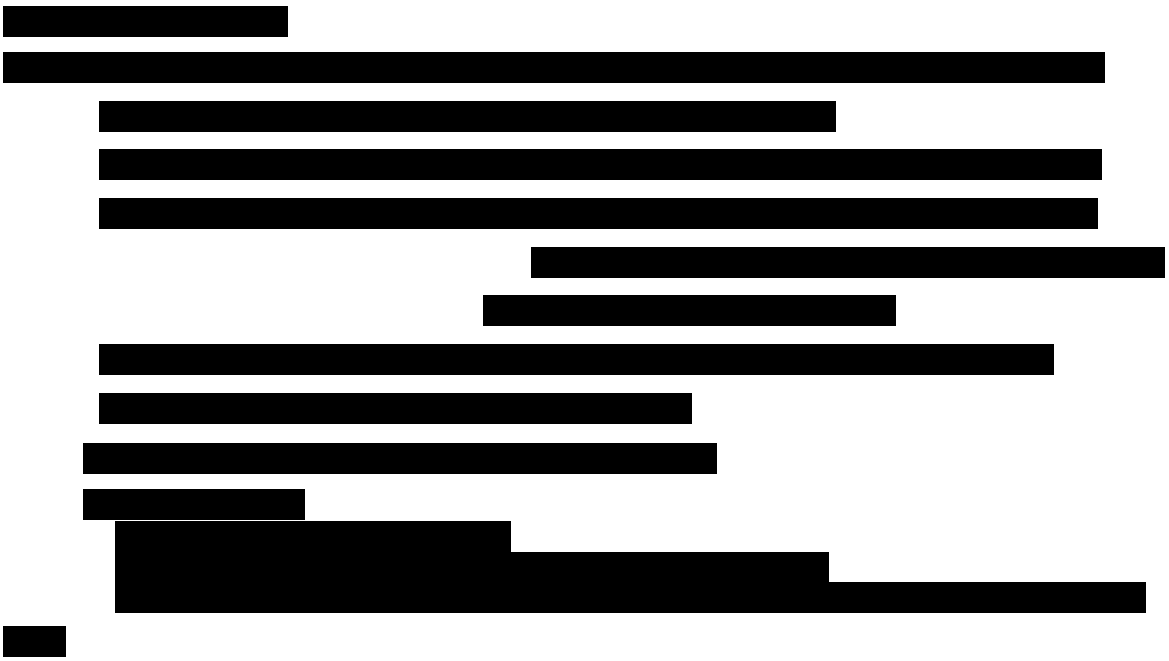
4. The proportion of subjects with worsening of stroke over the 48-hours/duration of admission:

Worsening of stroke is defined (i.e., Yes or No) as: progression, or hemorrhagic transformation, of the index stroke as documented by medical imaging and that is (a) life-threatening requiring intervention and/or (b) results in increased disability as gauged by a ≥ 4-point increase from lowest NIHSS pre decline and/or (c) results in death. Subjects with worsening of stroke will be identified based on a ≥ 4-point increase in their worst minus best NIHSS score and adverse events status (life threatening requiring intervention or death) as collected in the CRFs.

The simple effect of treatment on worsening stroke at 48 hours/admission duration will be examined by comparing the proportion of subjects who had worsening stroke (Yes or No) between the three treatments (RNS60 1.0 mL/kg/h, RNS60 0.5 mL/kg/h, and placebo 1.0 mL/kg/h) using GLM using residual pseudo-likelihood with a subject-specific expansion (RSPL)assuming a binary distribution. Covariates include the Age, baseline NIHSS, and baseline ASPECTS as binary factors used for block urn randomization, log of baseline infarct volume, baseline perfusion status, baseline occlusion location, and study site.

The predicted probability for each treatment group and LS mean difference between high-dose and placebo and low-dose and placebo groups along with the 95% CIs will be provided using the LSMEANS/DIFF statement. The comparison of the two combined active arms versus placebo will be performed using the LSMESTIMATE statement with the ILINK option. The odds ratio will also be calculated. The P-value for the hypothesis testing will also be provided. Treatment difference will be assessed with a 2-sided alpha level of 0.05.

The sample SAS code planned for the analysis is outlined below.



5. Barthel Index.

The effect of treatment on functional independence at Day 90 will be examined by comparing subjects achieving functional independence (Barthel Index score ≥95 as compared to <95) where subjects having a score ≥95 on the Barthel Index Score at Day 90 will be deemed to have achieved functional independence, and those scoring <95 at Day 90 will be deemed to have failed to acheive functional independence.

The proportion of subjects who achieved functional independence (BI score ≥95 vs. <95) at Day 90 will be compared between the three treatments (RNS60 1.0 mL/kg/h, RNS60 0.5 mL/kg/h, and placebo 1.0 mL/kg/h) using generalized linear mixed modeling using RSPL assuming a binary outcome (<95=0, ≥95=1). Covariates include the Age, baseline NIHSS, and ASPECTS binary factors used for block urn randomization and baseline BI total, log of baseline infarct volume, baseline perfusion status, baseline occlusion location, and study site. Treatment, visit day, and interaction of treatment and visit day are included as categorical fixed effects.

Sensitivity analysis of BI will be performed in a similar way to the primary analysis using the GLM approach with RSPL assuming a binary outcome ((<95=0, ≥95=1) where any missing value of BI will be imputed as functional independence not achieved (coded as 0).

3.4.3 Sensitivity Analysis for Secondary Efficacy Endpoints with Dichotomous Data for Baseline Collaterals

For each of the secondary efficacy endpoints described in section 3.4.2, additional sensitivity analysis will be performed using the GLM analysis approach where baseline collaterals will also be included in the model. For subjects with a baseline collateral score, the score will be converted to baseline collateral status. Baseline collaterals dichotomization will use the following conversion approach:

Dichotomization A (main): where a baseline collateral score of 0-2 indicates Baseline collateral status = Absent, and a baseline collateral score of 3-5 indicates Baseline collateral status = Present.

Dichotomization B: where a baseline collateral score of 0 indicates Baseline collateral status = Absent, and a baseline collateral score of 1-5 indicates Baseline collateral status = Present.

Dichotomization C: where a baseline collateral score of 0-1 indicates Baseline collateral status = Absent, and a baseline collateral score of 2-5 indicates Baseline collateral status = Present.

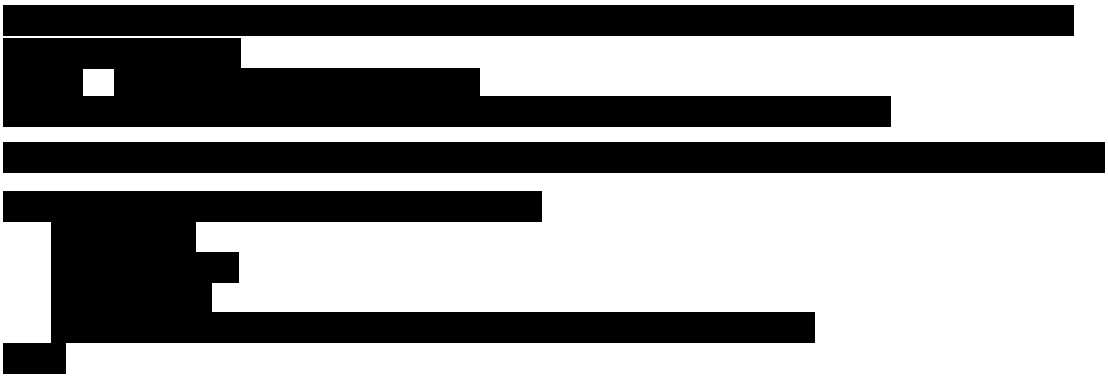
Each efficacy endpoint will be analyzed separately using the GLM model with the main dichotomization A approach included in the model. The SAS code for PROC GLIMMIX in section 3.4.2 will be modified to generate LS Means, LS Means Difference, 95% CIs, and odds ratio (as applicable). Separate GLM analysis with dichotomization B or dichotomization C may also be performed.

3.4.4 Additional Analysis

3.4.4.1 Analysis of mRS (0-6 scale)

An analysis will be performed using the nonparametric Wilcoxon test and the Mann-Whitney U test to determine if 90 day mRS (0-6 scale) differs between high-dose RNS60 (1.0 mL/kg/h RNS60) vs. placebo, low-dose (0.5 mL/kg/h RNS60) vs. placebo, and between combined dose vs. placebo.

The analysis will be carried out using the NPAR1WAY procedure in SAS where the model will include the mRS score at Day 90 as the analysis response variable, treatment as the CLASS variables, and will include the frequency of subjects within each mRS score category. The following SAS code and procedures will be performed using three sets of data: Placebo and low dose; Placebo and high dose; and Placebo and the two active doses considered as a single group. The sample SAS code is provided below:



The sum of scores and expected score under null hypothesis across each group will be displayed along with the one-sided p-value for each treatment vs. placebo. Additionally, an estimate of the probability that a random member of each treatment arm performs better than a random member of each placebo arm will be provided using the following steps:

1. Using the Wilcoxon scores, the following U-Statistic will be calculated for each arm:
   $U1 = R1 - [\frac{n1(n1+1)}{2}]$; where R is the sum of Ranks (sum of scores) for the treatment arm, and n1 is the number of subjects in the that arm.

2. The concordance probability will be calculated as follows: $f = \frac{U1}{n1n2}$; where U1 is the U-Statistic calculated above and n1 and n2 are the number of subjects in each arm.

3.4.4.2 Correlation Analysis between Infarct Volume and Clinical Outcomes

Correlation between infarct volume at different timepoints and clinical outcomes (mRS, NIHSS, BI) at 90 days will be measured using Spearman's correlation. Correlation will be performed for the high-dose RNS60, low-dose, and combined dose. Spearman's correlation coefficient and the corresponding p-value will be presented for each treatment.

The following outcomes will be evaluated:

- Correlation between infarct volume at 48 hours and mRS at 90 days
- Correlation between infarct volume at 48 hours and NIHSS at 90 days
- Correlation between infarct volume at 48 hours and BI at 90 days
- Correlation between infarct volume at 90 days and mRS at 90 days
- Correlation between infarct volume at 90 days and NIHSS at 90 days
- Correlation between infarct volume at 90 days and BI at 90 days

The sample SAS code is provided below:

[redacted]

3.4.4.3 Secondary Endpoints Analyses with Additional Covariates

The statistical models for one or more of the secondary endpoints may be amended to include additional covariates such as last known well time frame (≥6 hours or <6 hours) and use of thrombolytics. This may be performed as post-hoc analyses.

3.4.5 Biomarker Endpoints

The protocol specifies that blood will be collected for Ribonucleic Acid (RNA) and other biomarker analyses. Statistical treatment of these biomarkers is outside the scope of analyses to be performed for inclusion in the Clinical Study Report. Nonetheless, some specification of biomarker analysis may be desirable, and this is provided in Appendix D.

3.5 Safety Assessment

Safety data will be summarized by actual treatment received and in total based on the Safety Population.

3.5.1 Adverse Events (AEs)

An AE is defined as any untoward medical occurrence in a subject or clinical investigation participant administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment.

An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product. AEs will be captured from the start of study drug infusion through study completion (Day 90). All AEs will be coded to system organ class and preferred term using MedDRA version 24.0. A medical review of the coding reports will be performed prior to database lock. Treatment-emergent adverse events (TEAEs) are defined as AEs that start after the study drug infusion.

The investigator will grade the intensity of the AE as mild, moderate, or severe.

The relationship of an AE to study drug will be assessed as related, possibly related, or unrelated.

An overview of AEs will be provided including counts and percentages of subjects (and event counts) with the following:

- Any TEAEs (overall and by maximum severity)
- Any study drug related TEAEs (overall and by maximum severity)
- Any SAEs
- Any treatment-emergent serious AEs (TESAEs)
- Any study drug related TESAEs
- Any TEAEs leading to discontinuation of study drug infusion
- Any AEs leading to death
- TEAE occurrence by Month (Day 0-Day 7, Day 8 – Day 14, Day 15 – Day 21, Day 22 – Day 30, Day 31-Day 60, and Day 61-Day 90)

Counts and percentages of subjects (and event counts) will also be presented by system organ class and preferred term for each of the categories in the overview. If more than one event occurs with the same preferred term for the same subject, the subject will be counted only once for that preferred term using the most severe occurrence for the summary by severity. The number of events will be presented for overall category of TEAE and for each system organ class and preferred term. The number of events will not be presented for each category of maximum severity. AE listings will include the following information:

- Subject ID
- Adverse event term
- Adverse event preferred term
- System Organ Class
- Start date
- Outcome
- End date
- Relationship to study drug
- Action taken with study drug

- Severity
- SAE Criteria

AEs with missing start dates will be considered as treatment-emergent. The first date of the month will be used to impute missing start days and January will be used to impute missing start months.

Listings will be presented specifically for SAEs and TEAEs leading to discontinuation of study drug.

3.5.2 Clinical Laboratory Tests

Blood for clinical laboratory tests will be collected at baseline and Day 2 and processed by the local laboratory. A list of laboratory tests to be performed and their standard units is included in Appendix A. Normal range files for local laboratories used in the study are provided by each site one time prior to database lock.

Absolute values and changes from baseline in standard unit will be summarized descriptively. The incidence of abnormalities (as defined by normal ranges) and clinical significances prior to the study drug infusion and after the study drug infusion will be summarized with counts and percentages of subjects.

3.5.3 Vital Signs

Blood pressure and heart rate will be measured at baseline, and per routine site standard of care, every 8-12 hours during Day 1 and Day 2 until completion of the infusion. Temperature will be recorded at baseline only if collected as standard of care. Since blood pressure and heart rate are measured every 8 hours during Day 1 and Day 2, the timepoints for the descriptive summarization of blood pressure and heart rate will be derived as follows:

Table 6: Vital Signs Timepoints derivation

Derived Timepoint	Assessment Performed
Hour 8	After baseline and up to 12 hours
Hour 16	13 hours to 20 hours
Hour 24	21 hours to 32 hours
Hour 36	33 hours to 42 hours
Hour 48	43 hours and beyond

Absolute values and change from baseline through the end of drug infusion of BP, heart rate, and temperature will be summarized using descriptive statistics by treatment group and time point. The number of available observations and out-of-range values (see Table 7) will be presented by treatment group and time point. Vital sign variables will be listed. Hypotension and hypertension thresholds are site dependent and evaluated by the investigator based on the individual's medical history and current status.

The maximum deviation of BP from baseline between study drug and placebo control group to Day 2 will be analyzed by an analysis of covariance (ANCOVA) model with treatment group as a factor and baseline BP as a covariate. The least-squares means, standard errors, and the 2-tailed 95% CIs for each treatment group and for each comparison will be presented. The two-sided p-values testing for significance within treatment group change from baseline and comparisons between treatment groups will be presented.

Table 7: Vital Sign range for out-of-range values determination:

Variable	Range
Systolic Blood Pressure	90 to 140 mm Hg
Diastolic Blood Pressure	50 to 90 mm Hg
Heart Rate	60 to 100 beats per minute
Temperature	96.8 to 100.4°F (36°C to 38°C)

3.5.4 Electrocardiograms (ECG)

Electrocardiograms will be performed at baseline (within 4 hours of admission), 24h (±4 hours), and 48h, ±4 hours).

Absolute ECG values will be listed. ECG parameters (heart rate, PR interval, QRS duration, QT interval, and QT interval corrected for heart rate using Fridericia's correction (QTcF) interval) values and overall interpretation will be summarized by treatment group and time point. QTcF is the duration of the QT interval corrected for heart rate by Fridericia's formula: $QTcF=QT/RR^{1/3}$

The number and percentage of subjects in each of the following categories will be presented by treatment and in total:

- Post-baseline absolute QTcF interval >450 msec
- Post-baseline absolute QTcF interval >480 msec,
- Post-baseline absolute QTcF interval >500 msec.
- Change from baseline QTcF interval increase >30 msec,
- Change from baseline QTcF interval increase >60 msec.

Clinically abnormal and significant results will be flagged.

3.5.5 Physical Examinations

Physical examinations will be performed at baseline and every 8-12 hours during Day 1 and Day 2. The investigator will grade the result of the physical examinations as normal, not clinically significant abnormal, or clinically significant abnormal. All clinically significant abnormal findings from the physical examinations will be flagged in data listings.

Since physical examinations are measured every 8-12 hours during Day 1 and Day 2, the timepoints for the summarization of physical examinations will be derived in the same manner as the vital signs timepoints provided in Table 6 of this SAP.

3.5.6 Other Baseline and Safety Assessments

Other baseline and safety assessments such as Non-contrast Computed Tomography Scan (NCCT) brain and Computed Tomography Angiography (CTA), hospitalization, CXR, EVT,

Telemetry, and Pregnancy testing results will be listed. Clinically abnormal and significant results will be flagged.

4 DATA SAFETY MONITORING BOARD

A Data Safety Monitoring Board (DSMB) will monitor the safety of subjects over the course of the study. The DSMB will meet at least twice during the subject enrollment period to examine the unblinded accumulated safety data. Subjects, investigators, site staff and in general all personnel directly involved in the conduct of the study will remain blinded to the subjects' treatment assignment until the completion of the study.

Details related to the DSMB responsibilities, authorities, and procedures will be performed in accordance with the DSMB charter.

5 CLINICAL MONITORING OF DATA

To ensure overall quality in data capture and recording, the study will have experienced monitors conduct both on-site and remote monitoring visits at every site at a frequency/duration based on the rate of enrollment, site assessments and needs, and/or identified risks. Each site investigator agrees to allow the monitor direct access to relevant records, and to be available upon request to discuss any issues and to resolve queries. Monitoring responsibilities and expectations are detailed in the Clinical Monitoring Plan.

6 ANALYSIS TIMING

6.1 Interim Analysis

No interim analysis is planned.

6.2 Pre-Final Analysis

After the database is locked and exclusions from analysis populations have been finalized, the randomized treatment assignments will be unblinded and the pre-final analysis will be generated. Pre-final Tables, Figures and Listings (TFLs) will be provided approximately 3 weeks after database lock.

6.3 Final Analysis

After all comments on the pre-final analysis have been resolved and the study database is declared final, the final analysis will be generated. Final TFLs will be provided approximately one week after the study database is declared final. If there were no changes to the pre-final analysis or the study database, the pre-final TFLs may be considered final. In addition to TFLs, study data tabulation model (SDTM) data and analysis data model (ADaM) data along with associated files will be provided. Associated files may include annotated CRFs, SDTM specifications, SDTM programs, ADaM specifications, ADaM programs, TFL programs, and clinical data interchange standards consortium (CDISC) Define packages for both SDTM and ADaM data.

6.4 Changes from Protocol-Specified Statistical Analyses

The protocol specifies ITT population as all randomized subjects. This SAP clarifies that ITT population will include all randomized subjects who received study drug. ITT population will exclude subjects who were randomized and did not receive study drug.

There are no significant changes from protocol-specified statistical analysis apart from specifying the type of analysis to be used and further defining endpoints. The SAP will be considered as the governing document and in case of any differences between this SAP and the study protocol, the SAP will supersede the protocol.

6.5 Programming Specifications

Analyses will be performed using SAS® version 9.4 or higher. All available data will be presented in subject data listings which will be sorted by subject and visit date as applicable. Detailed Programming Specifications will be provided in a separate document.

APPENDIX A: CLINICAL LABORATORY TESTS

The tests detailed here will be performed as per local hospital laboratory.

Table 9: Protocol-Required Safety Assessments

Laboratory Tests	Parameters
Hematology	Platelet count (K/mm^3) Hemoglobin (g/dL) Hematocrit (%)
Chemistry	Blood Urea Nitrogen (mg/dL) Coagulation Panel (PTT and INR) Liver function test (LFT) panel ((ALT (U/L), AST (U/L) and Bilirubin (mg/dL)) Serum creatinine (mg/dL) Serum glucose (mg/dL)
Electrolytes	Sodium (mmol/L) Potassium (mmol/L) Chloride (mmol/L) Bicarbonate (mmol/L) Magnesium (mg/dL)
Pregnancy testing	Highly sensitive (serum or urine) human chorionic gonadotropin (β-hCG) pregnancy test (as needed for women of childbearing potential)

APPENDIX B: REFERENCES

Brott, T., Adams Jr, H. P., Olinger, C. P., Marler, J. R., Barsan, W. G., Biller, J., Spilker, J., Holleran, R., Eberle, R., Hertzberg, V. Rorick, M., Moomaw, C., & Walker, M., (1989). Measurements of acute cerebral infarction: a clinical examination scale. Stroke, 20(7), 864-870.

FDA (2018). Patient-Focused Drug Development Guidance Public Workshop: Methods to Identify What is Important to Patients & Select, Develop or Modify Fit-for-Purpose Clinical Outcomes Assessments, October 15-16, 2018.

Schlegel, D., Kolb, S. J., Luciano, J. M., Tovar, J. M., Cucchiara, B. L., Liebeskind, D. S., & Kasner, S. E. (2003). Utility of the NIH Stroke Scale as a predictor of hospital disposition. Stroke, 34(1), 134-137.

Shah, S., Vanclay, F., & Cooper, B. (1989). Improving the sensitivity of the Barthel Index for stroke rehabilitation. Journal of clinical epidemiology, 42(8), 703-709.

Zhao W and Weng Y. Block urn design - a new randomization algorithm for sequential trials with two or more treatments and balanced or unbalanced allocation. *Contemp Clin Trials*. 2011;32:953-61.

APPENDIX C: DEFINITION OF SAE

An SAE is defined as any untoward medical occurrence that, at any dose:
a. Results in death All deaths occurring during the follow up to Day 90 will be reported as an SAE. When reporting a death, the event or condition that caused or contributed to the fatal outcome should be reported as a single medical concept.
b. Is life-threatening The term 'life-threatening' in the definition of 'serious' refers to an event in which the participant was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death, if it were more severe.
c. Requires inpatient hospitalization or prolongation of existing hospitalization • In general, hospitalization signifies that the participant has been detained (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or outpatient setting. Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfills any other serious criteria, the event is serious. When in doubt as to whether "hospitalization" occurred or was necessary, the AE should be considered serious. • Hospitalization for elective treatment of a pre-existing condition that did not worsen from baseline is not considered an AE.
d. Results in persistent disability/incapacity • The term disability means a substantial disruption of a person's ability to conduct normal life functions. • This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, and accidental trauma (e.g., sprained ankle) which may interfere with or prevent everyday life functions but do not constitute a substantial disruption.
e. Results in a congenital anomaly/birth defect
f. Other situations: a SAE can also be an important medical event that may not result in death, be life-threatening, or require hospitalization, but may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition: • Medical or scientific judgment should be exercised in deciding whether SAE 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 jeopardize the participant or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These events should usually be considered serious.

- Examples of such events include 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.

APPENDIX D: POTENTIAL BIOMARKER ANALYSES

Exploratory analyses of biomarkers may be conducted in that subset of the ITT population consisting of subjects who consented to the collection of blood samples for RNA and other biomarkers. A list of the biomarker endpoints to be analyzed may be provided in the future.

Descriptive statistics for each endpoint may be tabulated by treatment arm and time (Day 1, Day 2 and Day 90).

Other possible analyses are as follows:

- To compare qualitatively those subjects who consented to biomarker collection versus the rest of the ITT population, descriptive statistics for important baseline characteristics (to be determined in the future) may be tabulated by subject cohort (those who consented to biomarker collection versus those who did not).
- The relationship between biomarkers and key clinical outcomes (mRS, NIHSS, BI and EQ-5D-5L) may be examined descriptively, as follows:
    - Within each treatment arm and for all treatment arms combined, scatterplots may be generated for each biomarker at Day 90 (horizontal axis) and each clinical outcome at Day 90 (mRS (scale 0-6, not dichotomized, NIHSS, BI, EQ-5D-5L; vertical axis). These may be presented individually or as one or more scatterplot matrices.
    - The Spearman rank correlation coefficients between each biomarker and each clinical outcome may be presented, either superimposed on the corresponding scatterplots as defined above, or tabulated in a separate table.
    - Irrespective of treatment, the empirical probability distribution function (ePDF) of each biomarker at Day 90 may be displayed graphically, one curve for each mutually exclusive subset of subjects as defined by the following cut points of the clinical outcomes at Day 90. For each biomarker, the ePDF curves would be overlaid on the same set of axes. (See Figure 9 of FDA (2018).) The ePDFs may be generated using the SAS UNIVARIATE procedure with the default KERNAL option.

        a. mRS: 0-2 (No disability), 3-6 (Disability). Thus, two curves may be overlaid—one for subjects with Day 90 mRS in the range 0-2 and the other for subjects with Day 90 mRS in the range 3-6.

        b. NIHSS: ≤ 5 (Mild), 6-13 (Adequate), ≥ 14 (Severe). Three curves may be overlaid.

        c. BI: The tertiles of the Day 90 total EQ-5D-5L score would be determined. The biomarker ePDF would be generated for the subjects in each tertile. Three curves may be overlaid.

        d. EQ-5D-5L: The tertiles of the Day 90 total EQ-5D-5L score would be determined. The biomarker ePDF will be generated for the subjects in each tertile. Three curves may be overlaid.