STATISTICAL ANALYSIS PLAN

NoNO, Inc.
Toronto, Ontario

Protocol NA-1-007

A Multicenter, Randomized, Double-blinded, Placebo-controlled, Parallel Group, Single-dose Design to Determine the Efficacy and Safety of Intravenous NA-1 in Subjects with Acute Ischemic Stroke Undergoing Endovascular Thrombectomy (ESCAPE-NA1 Trial)

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SIGNATURES OF APPROVAL

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LIST OF ABBREVIATIONS

AE	Adverse Event
AIS	Acute Ischemic Stroke
ANCOVA	Analysis of Covariance
CI	Confidence Interval
CRU	Clinical Research Unit (responsible for the Electronic Data Capture system)
CTA	Computerized Tomographic Angiography
CTP	Computerized Tomographic Perfusion
ASPECTS	Alberta Stroke Program Early Computerized Tomography Score
BI	Barthel Index
BNT	Boston Naming Test
DWI	Diffusion Weighted Imaging
ECG	Electrocardiogram
EMS	Emergency Medical Services
e-CRF	Electronic Case Report Form
FLAIR	Fluid Attenuated Inversion Recovery
ICH	Intracerebral Hemorrhage
ICA	Internal Carotid Artery
IDMC	Independent Data Monitoring Committee
IQR	interquartile range
ITT	Intent-to-Treat
MedDRA	Medical Dictionary for Regulatory Activities
MCA	Middle Cerebral Artery
MoCA	Montreal Cognitive Assessment
MRP	Magnetic Resonance Perfusion
mRS	Modified Rankin Scale
NCCT	Non-contrast Computed Tomography Scan
NIHSS	National Institutes of Health Stroke Scale
O-F	O'Brien-Fleming
PP	Per Protocol
SAE	Serious Adverse Event
SD	Standard Deviation
SNAP	Sunnybrook Hemispatial Neglect Procedure
SOC	System Organ Class
TEAE	Treatment-emergent Adverse Event
mTICI	Modified Thrombolysis in Cerebral Infarction scale
tPA	Tissue Plasminogen Activator

1.0 INTRODUCTION

This document provides the details of statistical analyses planned for Protocol No. NA-1-007. In addition, it discusses the statistical issues relevant to these analyses (e.g., sample data to be used and missing data).

1.1 Background

NA-1 is a synthetic, cell-permeant eicosapeptide (20 amino acids) that perturbs protein-protein interactions on the cytosolic surface of the cell membrane mediated by post-synaptic density 95 protein (PSD-95)[1], an abundant protein localized in post-synaptic densities of central nervous system neurons. It may provide significant benefit for the treatment of acute cerebral ischemia if administered to stroke patients who present to medical attention before infarction is complete. The rapid progression of irreversible brain injury in most acute strokes implies a short window of clinical efficacy of any treatment, including NA-1. The ability to identify patients with salvageable brain using the criteria used in the ESCAPE trial[2] provides an opportunity to target patients who may have the greatest benefit from neuroprotection, and to enhance further the impact of reperfusion therapies.[3] Our preclinical and clinical data support this notion.

The rationale for this study is as follows:

1. There is no convincing evidence from randomized controlled trials that neuroprotection can be of clinical benefit to patients with AIS. There is however, extensive preclinical evidence that neuroprotection is of greatest benefit for improving functional outcome in studies employing experimental animal stroke models in which stroke is followed by reperfusion, as compared with stroke models in which arterial vessel occlusion is undertaken without reperfusion.

2. The neuroprotectant, NA-1, has been demonstrated to be highly effective in reducing stroke size and improving the functional outcome of experimental animals subjected to acute stroke, including rats and primates. NA-1 is more effective in reducing infarct size and improving functional outcome in models of ischemia-reperfusion, as compared with permanent arterial occlusion.[4, 5]

3. NA-1 has an excellent safety profile in preclinical animal studies, a human Phase 1 trial, and a human Phase 2 study (the ENACT trial),[6] Patients in the ENACT trial were also individuals who were being subjected to an endovascular procedure (intracranial aneurysm repair) and to treatment with NA-1 or placebo.

4. The ESCAPE and other recent trials of endovascular thrombectomy for AIS have demonstrated that modern acute endovascular therapy can rapidly recanalize blocked major arteries in patients with acute stroke arising from large artery occlusion with a low complication rate and high complete reperfusion rates.[2, 7-9] This paradigm replicates the preclinical animal ischemia-reperfusion models in which neuroprotectants have the highest demonstrated efficacy.

5. NA-1 has shown promising results in reducing ischemic brain damage in humans having demographics similar to those of stroke patients.[6]

6. There is a compelling need to develop neuroprotectants in order to increase the proportion of patients who may benefit from recanalization therapies. These agents could improve the outcomes of those who receive endovascular recanalization and make more patients into candidates for endovascular or pharmacological recanalization treatment.

1.2 Objectives

1.2.1 Primary Objectives

The primary objective is to determine the efficacy of the neuroprotectant, NA-1, in reducing global disability in subjects with major acute ischemic stroke (AIS) with a small established infarct core and with good collateral circulation who are selected for endovascular revascularization.

1.2.2 Secondary Objectives

The secondary objectives are to determine the efficacy of NA-1 in:

- Reducing functional dependence
- Improving neurological outcome
- Improving activities of daily living
- Reducing mortality rate

1.2.3 Tertiary Objectives

The tertiary objectives are to determine the efficacy of NA-1 in:

- Improving health-related quality of life.
- Decreasing infarct volume at follow-up imaging
- Improving cognitive function

1.2.4 Leading Safety Objectives

The leading safety objectives are to determine the effect of administering a dose of 2.6 mg/kg (up to a maximum dose of 270 mg) intravenous (IV) infusion of NA-1 to subjects with acute stroke who are selected for endovascular revascularization on the incidence of serious adverse events (SAEs) and 90-day mortality.

1.3 Study Design

This is a Phase 3 randomized, multicenter, blinded, placebo-controlled, parallel group, single-dose design. Per inclusion/exclusion criteria, subjects suffering an acute stroke who are selected for endovascular revascularization in accordance with local institutional practices and who present with a small established infarct core with good collateral circulation, will be given a single, 2.6 mg/kg (up to a maximum of 270 mg) intravenous dose of NA-1 or placebo. Ideally, dosing should begin within 30 minutes of randomization and within 60 minutes of the NCCT.

A total of 1120 subjects aged 18 years and older will be enrolled into the study. A patient who consents but is not randomized will be considered a screen failure. A subject is considered randomized the moment the randomization process is completed on-line. This is time “0” for the study. All subjects will be followed for 90 days (or until death if prior to 90 days). Subjects who are randomized but do not receive study drug will still be followed through the 90-day study period. The end of study is defined as the date that the last enrolled subject has completed their Day 90 visit.

The Schedule of Assessments is presented in Table 1.

1.4 Sample Size Determination

Assuming a 52% overall responder rate for the placebo group, there will be 81.7% power to detect an 8.7% absolute effect difference between response rate (proportion of responders, with Day 90 mRS in the range 0 to 2) with NA-1 and placebo, at alpha level 0.05, 2-sided with a planned sample size of 1076 evaluable subjects, randomized 1:1, per group, accounting for a single interim analysis when 600 subject have reached their primary endpoint assessments, with O'Brien-Fleming alpha-spending function (EaST® V6.3) stopping boundary for overwhelming efficacy and a non-binding 1% conditional power futility stopping boundary. (See Section 3.0 for further details on the interim analysis.) The sample size will be inflated approximately 4% to N= 560 per group to account for loss-to-follow-up and dropouts.

1.5 Randomization

Treatments (placebo vs. NA-1) will be assigned 1:1 by application of a stratified minimization algorithm administered through a secure web-based, real-time interaction with the site and the central server. The time of randomization on the central server will be considered baseline (i.e., time zero) for the study. Study drug is intended to be infused within 30 minutes post-randomization and within 60 minutes of the NCCT. Although each vial of NA-1 or placebo will have a unique ID number that will be linked to the randomization code generated by the minimization algorithm, the vials will appear to be identical to those dispensing them and to the subjects. Thus, individuals at the sites will be blinded to the treatment assignments.

In order to balance the treatment assignment among subjects receiving different combinations of adjunct thrombolytic and thrombectomy therapies, randomization will be stratified by (1) intravenous thrombolysis with alteplase (yes/no) and by (2) a subject's initial thrombectomy approach (stent retriever vs. thrombus aspiration device). Planned initial use of any other endovascular device type or intra-arterial medications will constitute an exclusion criterion. Similarly, if intravenous thrombolysis is planned for the subject, use of any intravenous thrombolytic other than alteplase is an exclusion criterion. Thus, there will be 4 randomization strata:

a. Alteplase+stent retriever
b. Alteplase+thrombus aspiration device

c. Stent retriever without Alteplase

d. Thrombus aspiration device without Alteplase

Randomization will be by the minimally sufficient minimization procedure of Zhao.[10] The procedure will be implemented to achieve balance on 6 baseline subject-level prognostic variables: age, sex, baseline NIHSS scores, baseline ASPECTs score, occlusion location and study site. At the time of randomization, subject age will be calculated against the central server date and time, which will be synchronized weekly to the Denver atomic clock. This overall approach will ensure that the subjects entered into the trial will be matched on the six key prognostic covariates, within strata and between treatment arms, thereby minimizing likelihood of chance confounding of the estimates of the treatment effects with covariate effects. In addition to ensuring a balanced analysis at the conclusion of the study, the minimization will also provide some assurance that adequate balance will be maintained at the time of interim analysis.

The randomization of the first 40 subjects will be completed using a simple random number generator to yield an unstratified randomization, with each subject having an equal probability of assignment to either treatment arm. Thereafter, randomization will be stratified as described above, with the minimization algorithm applied to achieve balance simultaneously across the covariates.

All subjects who are randomized will be accounted for in the trial database and followed rigorously for the primary and key secondary outcomes to minimize issues of imputation/missing data. The randomization number and time will be automatically printed from the randomization website and it will be transmitted to the central database to create the case in the electronic case report form (e-CRF). The randomization date, time and the two stratification variables, and the 6 covariates for the minimization algorithm will not be editable once the subject is randomized. Once a subject is entered into the randomization website, that subject's e-CRF will be auto-created and ready for data entry.

The automated system will inform site staff when re-stocking of the refrigerator containing study drug is required and will specify the vial numbers to be restocked. This will ensure that a blinded investigator cannot match a vial that was just given to a subject to a single re-stocked vial and adds to the assurance of the study blind.

Re-stocking will take place within one business day per the local participating site's working practice.

All subjects, investigators, their clinical staff, the clinical coordinating center, the data management group, and the sponsor staff and delegates will be blinded to the randomization codes. The local laboratories will also be blinded.

Table 1. Schedule of Assessments

++	Baseline	Post- EVT (~2 h)	Day 1 (24 ± 12 h from random-ization)	Day 2 (48 ± 8 h from randomization)	Day 5 or discharge (±1 d)	Day 30 (±5 d)	Day 90 (±14 d)
Informed consent	X						
Attempt at regained capacity informed consent★			X	X	X	X	X
History and examination	X						
Weight	X*				X (Actual)		
Vital Signs (BP, HR, Temp) ★★	X	X	X	X	X		
Randomization/ Study drug administration	X						
Mortality					X	X	X
NIHSS	X	X	X	X	X	X	X
mRS			X¶		X	X	X
Barthel Index	X					X	X
BNT15, SNAP, MoCA¶¶							X
EQ-5D-5L						X	X
NCCT head	X						
CTA (Circle of Willis)	X						
Endovascular Procedure	X						
MR head			X**				
CBC, electrolytes, INR, aPTT, serum creatinine and serum glucose	X‡		X				
Pregnancy test	X‡‡						
Immunogenicity sample‡‡‡			X			X	
Pharmacokinetic samples‡‡‡‡	X						
ECG	X‡		X				
AE assessment	Collected to Day 30 visit						
SAE assessment	Collected to Day 90 visit						
Prior medications	X§§						
Concomitant medications	Collected to Day 30 visit						

★ If the original process involved anyone other than the subject(and if required), site staff will make ongoing efforts until (1) regained capacity consent is obtained from subject, (2) death, or (3) completion of the Day 90 assessment.

★★ Vital signs (BP, HR only) should be recorded immediately before and after completion of the study drug infusion, temperature will be taken at baseline per standard of care .¶ Historical (pre-stroke) score

¶¶ Assessments will be conducted only if the tool/scale is available in local language.

* At baseline estimated or actual weight

** Day 1 MR head may be supplanted by an NCCT head if MR is unavailable or contraindicated.

‡ ECG testing and Blood should be drawn at baseline, but results are not required prior to randomization. For ECG, if treating physician deems that pre-treatment ECG impedes access to timely care, ECG must be completed within 6 hours of randomization.

‡‡ If the subject is female and is of childbearing potential a pregnancy test (urine or serum point-of-care pregnancy test) must be completed and the result must be negative; this is the only mandatory laboratory test prior to randomization

‡‡‡ Immunogenicity samples will be taken from a subset of up to 250 subjects at Canadian and US sites.

‡‡‡‡ PK samples will be taken from up to 100 subjects at a subset of Canadian and US sites. Samples will be taken at pre-dose and at 10, 20, 30, 60 and 120 min after the start of drug administration.

§§ Prior medications should be documented but their documentation not required prior to randomization.

d = days; h = hours

1.6 Blinding

The study is conducted in a blinded manner. All subjects, investigators, their clinical staff, the clinical coordinating center, the data management group, and the sponsor staff and delegates will be blinded to the randomization codes. The local laboratories will also be blinded.

The IDMC reports and analyses for Closed Sessions will be organized by treatment arm ("unblinded"). In order to ensure confidentiality and minimize bias, the information will be provided to the IDMC by a group that is independent of the sponsor and blinded project team implementing the trial. A firewall will be maintained between the IDMC (unblinded) and the project staff (blinded). The IDMC will review safety data throughout the trial on an ongoing basis, and other data as requested by the IDMC, in addition to the interim analysis results.

The person responsible for the study drug labelling will be unblinded, as will the independent statistical group preparing the reports for the IDMC. The person responsible for the data management group, who manages the programming of the randomization system will be unblinded. This individual will be the contact person in the event that unblinding is necessary. This individual will not participate in data management and will only communicate unblinded data as set out below when contacted by the medical monitor.

Otherwise, randomization data will be kept strictly confidential, accessible only to authorized persons, until the time of unblinding after data lock at the time of interim analysis and at end of the study.

In case of emergency, a rapid unblinding procedure is available to investigators. If the investigator decides that the treatment code needs to be broken in the interest of subject safety, the investigator will have direct access to the ESCAPE-NA1 CRU center to request unblinding of the specific subject. The CRU will respond in writing to the investigator only with the unblinded patient treatment allocation.

Only the investigator requesting the unblinding will receive the unblinding information. The investigator will promptly inform the Sponsor when a request to unblind is made and the circumstances involved. Any case that is unblinded in this way will be documented in a blinded manner in central files.

1.7 Definitions

Baseline: A subject's baseline value for a given endpoint or parameter is defined as his/her latest measurement taken prior to study drug administration.

Prior and Concomitant Medications: Prior medications are defined as those taken within three days of treatment initiation. Concomitant medications are defined as those taken during study drug administration or after study drug has been administered through and including the Day 30 visit. All prior and all concomitant medications will be recorded on the electronic case report form (e-CRF).

Randomization: The time of randomization is defined as the time randomization occurred on the central server and this time is considered time zero for the study. All time windows are calculated from the time of randomization.

Treatment-Emergent Adverse Event: A treatment-emergent adverse event (TEAE) is one that first occurs or worsens in severity or frequency after study drug has been administered through to and including the Day 30 visit. SAEs are collected through the last study visit. Those AEs that start at the same time and date as the study drug administration and those that first occur or worsen after the start of study drug administration will be considered TEAEs.

AEs with partial or missing dates will be handled as follows:

- If the start day and/or month of the AE is missing and the AE in question occurs concurrently or subsequent to the study drug administration, the AE will be considered treatment-emergent.
- If the start date is completely missing then the AE will be considered treatment-emergent.

2.0 ANALYSIS POPULATIONS

2.1 Intent-to-Treat Population

The primary efficacy analysis will be conducted on the ITT population, defined as all subjects randomized into the trial with grouping by randomized treatment, regardless of treatment actually received. Deceased subjects will be included in the ITT population with a mRS score of 6, NIHSS of 42 and Barthel Index of 0. An ITT analysis will also be conducted for the secondary endpoints, with subjects grouped according to the randomized (intended) treatment.

2.2 Safety Population

The safety population comprises all subjects receiving any amount of study drug. In safety analyses, subjects will be grouped according to treatment actually received.

2.3 Per Protocol Population

The primary analysis will be repeated on the Per Protocol (PP) population, defined to be all subjects randomized and treated, with no major protocol deviations. We define "major protocol deviations" as those with the potential to bias, confound, or otherwise obscure the treatment effect estimates or which involve ethical standards. This population will be determined via a blinded review of protocol deviations at the end of the trial before database lock and unblinding.

Missing data due to death during the study will not exclude a patient from the PP population (i.e. death is not considered a major protocol violation).

Prior to unblinding, the imaging from each subject at the time of inclusion will be adjudicated to determine whether they have met the criteria for endovascular intervention, and hence for the trial. This will include review of baseline NCCT, CTA and if performed CTP or MRP. Subjects who do not meet the imaging criteria outlined in the trial inclusion/exclusion criteria will be reviewed in a blinded fashion as described above to determine if they meet the criteria for a "major protocol deviation"..

3.0 INTERIM EFFICACY ANALYSES

An efficacy interim analysis after approximately 600 subjects complete the Day 90 follow-up (~56% information) will be conducted by the unblinded statistician in the independent Statistical Group (ISG) using the alpha spending function method[11] with O'Brien and Fleming[12] type stopping boundary for efficacy and a non-binding conditional power boundary (based on the observed trend at the interim analysis) for futility. Overwhelming efficacy at the interim analysis would occur if the test statistic crosses the O-F superiority boundary. For an interim analysis conducted at exactly 55.8% information, the superiority critical p-value for stopping (boundary value) would be 0.003 and that at the end of the study (primary analysis) would be 0.024, 1-sided. Conversely if the conditional power estimate based on the observed trend at the interim is <1% the trial may be deemed to be futile, contingent on consideration of other safety and efficacy factors (i.e., the futility boundary is nonbinding).

The IDMC may recommend stopping for futility at the interim analysis if the test statistic crosses the O-F or conditional power boundary. However, notwithstanding the O-F superiority critical p-value of 0.003 for stopping (boundary value) at the interim analysis of the primary efficacy outcome, the IDMC will be instructed that it should not recommend to stop the trial for overwhelming efficacy unless otherwise instructed by the Steering Committee at the Open Session of the IDMC meeting. The IDMC Charter will provide further details on the rationale for, and how, these recommendations will be communicated.

To prevent operational bias all interim results on safety and efficacy will be reported only to the IDMC, keeping the sponsor, project team, investigators and subject blind to results by treatment assignment during the study. Firewalls will be in place at the Statistical Group preparing all interim reports to protect and sequester all interim results on safety and efficacy

4.0 DATA REVIEW

Relevant past medical history as well as prior and concomitant medications will be listed. AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) to assign a system organ class (SOC) and preferred term to each AE.

When the database has been declared complete and accurate, the database will be locked and subsequently unblinded.

5.0 MISSING DATA AND DATA TRANSFORMATION

5.1 Missing Data

Every effort will be made to keep missing data, particularly the Day 90 outcome assessments, to a minimum. However, some missing data may be inevitable due to, for example, loss to follow-up. This was kept to a minimum of 1.3% in the ESCAPE trial.[2] Deceased subjects will be assigned scores of 6 on the mRS, 42 on the NIHSS, 0 on the Barthel Index and be counted as non-responders. For the primary analysis for regulatory submission, we will assume that subjects who are missing the primary endpoint data will be considered non-responders. If more than 5% of subject randomized are missing the primary endpoint, two sensitivity analyses will be employed to examine alternatives to this extreme single-value imputation: (1) multiple imputation of the ordinal mRS score which will then be converted to the corresponding binary score; and (2) if, as expected, ≤ 5% of the mRS data are missing, a completers' analysis.

5.2 Data Transformation

No transformation of the data is planned other than for volume of stroke. Note that stroke volume data are often dispersed and highly non-normal and can be normalized with an inverse cubic root transformation. Alternate Box-Cox power transformations may be required, depending upon the data distribution (note: the inverse cubic root transformation is a member of the Box-Cox power series). No data transformation is planned for the primary analysis.

6.0 STATISTICAL METHODS

The software used for all summary statistical analyses will be SAS® (SAS Institute, Inc.) version 9.4 or later.

Unless otherwise noted, categorical data will be summarized for each treatment group using counts and percentages, with the denominator for percentages being the number of subjects in the population of interest. Unless otherwise noted, continuous data will be summarized for each treatment group using the number of observations (n), mean, standard deviation (SD), median, minimum, and maximum. Some continuous data will be reported as the median, interquartile range (IQR), minimum and maximum according to the clinical meaning of the data.

Percentages will be rounded to one decimal place, except 0% and 100% will be displayed without any decimal places. Minima and maxima will be rounded to the precision of the original value; means and medians will be rounded to one decimal place greater than the precision of the original value; SDs will be rounded to two decimal places greater than the precision of the original value. P-values will be reported to four decimal places (0.xxxx), with values less than 0.0001 presented as <0.0001.

Inferential analyses will generally include statistics such as 2-sided 95% confidence intervals (CI), and p-values. Unless stated otherwise, all statistical tests will be 2-sided hypothesis tests performed at the 0.05 level of significance.

In order to protect the overall trial false positive rate, the primary efficacy analysis, key secondary outcome analysis and secondary endpoints will be analyzed in a fixed sequence, stopping and calling all subsequent analyses exploratory (and accepting the null hypothesis) at the first failed hypothesis test in the sequence. The fixed sequential order is:

1. Primary efficacy outcome
2. Key secondary outcome analysis (shift analysis using proportional odds model);
3. Secondary efficacy endpoints, as specified in the order presented below in Section 6.7.

All data will be included in subject data listings for all randomized subjects.

A final, unblinded, statistical report that will include both efficacy and safety evaluations will be generated upon completion of the trial. The final report will be distributed to the Trial Steering Committee

6.1 Subject Disposition

Subject disposition will be summarized and tabulated for ITT, PP and Safety populations. The summaries will include the number and percentage of subjects that completed the study and those that terminated early from the study (i.e., left the study prior to the Day 90 visit). Early terminations will be categorized by the reason for study discontinuation. Death will not be considered early termination.

In addition, a tabulation of the number and percentage of subjects randomized will be summarized by site for the ITT population.

6.2 Protocol Deviations and Protocol Violations

The case report form pre-specifies the major protocol violations and protocol deviations. The data will be reviewed regularly to identify both. Major protocol deviations will be determined during a data review meeting prior to database lock. These may include but may not be limited to:

- Enrollment did not comply with inclusion criteria
- Enrollment did not comply with exclusion criteria
- Consent not obtained.
- Subjects who did not receive a per-protocol dose (none or incomplete dose), or received an incorrect study drug vial, or received the study drug infusion over 15 or more minutes.

The number and percentage of subjects in the ITT population with protocol violations will be summarized by treatment group and overall.

6.3 Treatments

Per protocol, an IV solution of 20mg NA-1/ml will be given to subjects with a body weight < 105 kg to achieve a final target dose of 2.6 mg NA-1/kg. Subjects weighing 105-120 kg will each receive a total dose of 270 mg of study drug (i.e., the entire 13.5 ml contained in the study drug vial). However, per-protocol, at the time of dosing, the total dose is based on the subject's estimated weight if an exact weight is not available. A second weight, using an in-hospital scales, will be obtained by day 5 or discharge (±1 d). Discrepancies between the weight estimate and actual weight obtained using in-hospital scales may result in some subjects weighing >105 Kg receiving less than 270 mg, and some subjects weighing < 105 Kg receiving the full 270 mg dose. Study drug monitors at each site will record the total volume of NA-1 solution received by each subject and their estimate of body mass at the time of treatment. Two measures of subjects' NA-1 exposure and 4 additional subject-level measures of the timeliness of the interventions will be computed as follows.

1. Number subjects who received any NA-1
2. Descriptive statistics for:
   a. Time from randomization to start of NA-1 infusion
   b. Time from NCCT to start of NA-1 infusion
   c. Subject's Actual exposure (mg NA-1/kg body wt) =
      [(20mg NA-1/ml of IV solution)*ml dose received]/kg body wt, where "kg body weight" was obtained using an in-hospital scale.
   d. Subject's Relative exposure (%) =
      (Actual exposure*2.6 mg NA-1/kg body wt) *100. where "kg body weight" was obtained using an in-hospital scale.
   e. Time from NCCT to 1st recanalization
   f. Time from NCCT to arterial access puncture

An additional 7 measures of overall dosing compliance and timeliness of intervention, at the treatment group level, will be computed from the subject-level measures and reported as percentages of the number of subjects in each treatment arm who received NA-1 therapy or placebo.

3. Overall relative NA-1 dosing compliance (%)= 100*[A+B]/C where A = number of subjects who received 2.6 mg NA-1/kg body wt; B = number of subjects who received 270mg total dose; C = number of subjects who received any amount of NA-1. As dosing compliance (per protocol) is the subject of this calculation, "kg

body wt" in this instance will be the weight (actual or estimated) obtained at the time of dosing.

4. Speed of intervention metrics measured on NA-1 and placebo treated subjects (% of each treatment group):
   a. % of subjects with time from randomization to NA-1 or placebo infusion < 30 minutes
   b. % of subjects with time from NCCT to infusion with NA-1 or placebo infusion < 60 minutes
   c. % of NA-1 and placebo subjects with time from NCCT to recanalization < 75 minutes
   d. % of NA-1 and placebo subjects with time from NCCT to recanalization < 90 minutes
   e. % of NA-1 and placebo subjects with time from NCCT to arterial access puncture <45 minutes
   f. % NA-1 and placebo subjects with time from NCCT to arterial access puncture <60 minutes

All measures will be summarized separately for the ITT, PP, and safety populations; individual exposures and speed of intervention times will be listed by treatment arm and subject.

6.4 Demographic and Baseline Characteristics

Subject demographic and baseline characteristics will be summarized with descriptive statistics for each treatment group. Demographic variables include, but are not limited to: age, sex, race-ethnicity, weight at time of dosing (in kg), and weight as determined in hospital (in kg). Baseline characteristics include but are not limited to: prior use of alteplase (tPA), intended first thrombectomy approach (stent retriever or thrombus aspiration device), baseline NIHSS score, baseline ASPECTS score, occlusion location, and site. The summaries will be provided for both the ITT and Per Protocol populations. Inferential statistics (i.e., p-values or CI) will not be provided for these data with the exception of the results of a Fisher's Exact Test which will be performed for the two stratification variables "alteplase use" (yes/no), and "first declared thrombectomy device" (stent retriever / aspiration device) in order to assess balance across treatment groups.

6.5 Pooling of Sites

In order to avoid sparse sites (sites with fewer than 5 randomized subjects) and the potential for too many levels for the explanatory variable of "site", sites within a

geographic region (Canada/USA/Rest of World) with fewer than 5 randomized subjects will be pooled into a single pooled site for use in efficacy analyses. If a resulting pooled site still has fewer than 5 randomized subjects, it will be further pooled with the smallest remaining unpooled site from that geographic region. If the resulting pooled site still has fewer than 5 randomized subjects, it will be further pooled with the smallest pooled site from another geographic region. This pooling is estimated to make up approximately 15% of all enrolled subjects by the time that the first 600 subjects will have been enrolled.

7.0 EFFICACY ANALYSIS

Efficacy analyses are summarized in Table 2.

7.1 Primary Outcome Variable Analysis

7.1.1 Primary Outcome

The primary endpoint used in this trial will be global disability as measured by the mRS at Day 90. Scores for the mRS range from 0 to 6, with 0 indicating no residual symptoms; 5 indicating bedbound, requiring constant care; and 6 indicating death. The mRS will be obtained at Day 5 (or discharge) and Days 30 and 90. Premorbid mRS status will also be obtained retrospectively at 24 hours.

The primary efficacy analyses will be conducted in the ITT population, defined as all subjects randomized into the trial with grouping by randomized treatment, regardless of treatment actually received. Deceased subjects will be included in the ITT population with a mRS score of 6. The primary endpoint used in this trial will be global disability, as measured by the mRS, at Day 90.

7.1.2 Statistical Hypothesis

The primary hypothesis is:

$$H_0: \pi_{NA\text{-}1} = \pi_{placebo} \text{ VS} \qquad H_a: \pi_{NA\text{-}1} \neq \pi_{placebo}$$

Where $\pi_{NA\text{-}1}$ and $\pi_{placebo}$ are the NA-1 and placebo population proportions of responders, defined as subjects whose Day 90 mRS score is ≤ 2.

7.1.3 Primary Efficacy Analysis

The primary efficacy outcome is the overall proportion subjects experiencing a favorable functional outcome 90-days post-randomization; where favorable is defined as a mRS score of 0, 1, or 2. Subjects with favorable functional outcomes are defined as responders. Subjects missing the day 90 primary assessment for any reason will be considered non-responders for the primary analysis.

The primary hypothesis to be tested is that administration of NA-1 will result in an increase in the proportion of responders (as defined by a score of 0-2) at Day 90. The primary analysis will be a Wald test for treatment group difference in the primary outcome from a logistic regression adjusted for the 2 stratification variables (alteplase use, first declared thrombectomy device), and the 6 covariates used in the minimization (age, sex, baseline NIHSS score, baseline ASPECTS score, occlusion location, and site). This will be conducted on the ITT population at the 2-sided 0.05 significance level overall (for the trial), adjusted for the interim analysis per the O'Brien-Fleming boundary spending function.

The following SAS PROC LOGISTIC code will be implemented for the binary primary endpoint analysis:

```
proc logistic data=adqs;
   where ittfl = 'Y' and qstest = 'mRS (binary)';
   class occloc alteplase thromb_app site
         trtp(param=ref ref='Placebo') ;
   model mRS = age sex blnihss blaspect occloc
               alteplase*thromb_app site trtp;
   oddsratio trtp;
run;
```

Where mRS is the binary independent functioning score made by dichotomizing mRS at 2: mRS≤2 = independent functioning. Occloc is the occlusion location (MCA vs. ICA), blnihss is the baseline NIHSS score (0-42), blaspect is the baseline ASPECTS score (0-10), alteplase is an indicator for having received IV alteplase treatment, thromb_app is the thrombectomy approach (stent retriever vs. aspiration device), alteplase*thromb_app is a 4-level stratification, site is investigative site, sex and age are subjects' sex (male/female) and age (years) at study entry, and trtp is the planned treatment.

All odds ratios (with Wald 95% C.I.s) and corresponding Wald test statistics will be summarized in a table, by select subpopulations. Actual proportions with and without independent functioning will be reported as will the proportions in each of the ordinal mRS categories.

Three supportive analyses to the primary analysis will be conducted: (1) the primary analysis reapplied to the Per Protocol population; (2) a re-randomization analysis to demonstrate that minimization did not bias the primary endpoint analysis. The later test will consist of rerunning the randomization 5,000 times on the original subjects' data and then performing the primary endpoint analysis on each of the re-randomized data sets. This will yield a distribution of 5,000 p-values that will correspond to the p-value distribution expected under the null hypothesis of no NA-1 effect. The p-value from the actual primary analysis will be compared to the distribution of randomization p-values. If (for example) the minimization was unbiased and actual p-value was 0.01, we would expect P=0.01 to be near the 1st percentile of the distribution of the 5000 p-values. A variant of this methodology was successfully used to validate the minimization in the ESCAPE trial.[2] (3) The primary analysis will be repeated on the PP population using actual first endovascular approach (instead of declared approach).

7.2 Secondary Efficacy Analyses

Descriptive summaries will be provided for each secondary endpoint in the ITT population. As with the primary endpoint, secondary endpoint analyses will also generally be adjusted for both the stratification variables and covariates used in the minimization algorithm.

All tests will be conducted with two-sided level of significance alpha = 0.05. A fixed sequence multiple testing procedure will control the overall experiment-wise error rate for the trial (see below). It pre-specifies that, with all tests conducted at the same pre-specified significance level, the primary endpoint will be tested first, and all subsequent tests are considered failed and deemed exploratory if conducted, in the order specified (primary analysis first, key secondary analysis second, etc.), after the first test which fails. All tests that follow the first failed test, are considered exploratory. For the purpose of clarity, since the key secondary analysis of the ordinal mRS scores will employ a proportional odds model (POM), if test of the proportional odds assumption shows the assumption to be invalid, this key secondary analysis will not be performed, and the remaining secondary tests will still be considered to be protected.

The secondary endpoints, and the order they are to be tested, are:

1. Shift to reduced functional dependence analyzed across the whole distribution of scores on the mRS at Day 90 or the last rating.

2. Proportion of subjects with good neurological outcome, as defined by a score of 0-2 vs 3 or greater on the NIHSS at Day 90 or the last rating.

3. Proportion of subjects with functional independence in activities of daily living, as defined by a score of ≥ 95 vs 0-90 on the Barthel Index at Day 90 or the last rating.

4. The proportions of control and NA-1 treated subjects who die by study day 90.

5. The proportion of subjects with mRS score of 0-1 at Day 90.

7.2.1 Key Secondary Outcome Analysis

A key secondary analysis of the ordinal mRS scores will employ a proportional odds model (POM) to test the hypothesis that, among randomized subjects, those who are treated with NA-1 will show a shift in their mRS score distribution at 90 days relative to the mRS distribution of the placebo subjects. The magnitude of the shift will be estimated as the common odds ratio (95% C.I.). Modified Rankin scores of 5 and 6 (bed-bound with severe disability, and death) will be collapsed into a single category representing severely limited functioning. A covariate-adjusted POM will be used to derive the common odds of improvement (i.e. the NA-1 vs. placebo "shift" in mRS score distributions). Model covariates will include all of the variables in the minimization algorithm, both stratification variables and covariates.

The following example SAS code can be used to fit the POM to the subjects' mRS score:

```
ODS graphics on;
proc logistic data=adqs;
   where ittfl = 'Y' and qstest = 'mRS (Ordinal)';
   class occloc alteplase thromb_app site
         trtp(param=ref ref='Placebo') ;
   model mRS = age sex blnihss blaspect occloc
               alteplase*thromb_app site trtp;
   effectplot interaction(x=trtp sliceby=mRS)/polybar;
   oddsratio trtp;
run;
```

Where "mRS' is the collapsed mRS scale values ranging from 0 to 5 (level 5 = 5+6 mRS combined), "occloc" is the occlusion location (ICA vs. MCA), "blnihss" is the baseline NIHSS score (0-42), "blaspect' is the baseline ASPECTS score (0-10), 'alteplase' is an indicator for having received IV alteplase treatment, thromb_app is the thrombectomy approach (stent retriever vs. aspiration device), alteplase* thromb_app is a 4-level stratification, "site" is investigative site, subject sex and age are the subjects' sex and age at study entry, and "trtp" is the planned treatment (NA-1, placebo).

When SAS fits the POM, it runs a global test for a shift across all 6 mRS categories in the NA-1 group relative to the placebo; this is the test of the proportional odds (PO) assumption. The proportional odds assumption will be checked via the score test at an alpha-level of 0.15 and also using graphical methods to view the cumulative log odds for each mRS score. If the assumption holds, the POM estimates a single fixed odds ratio for the 5 cumulative binary endpoints defined as follows:

a. The proportion of subjects with mRS = 0 vs. the proportion with mRS>0
b. The proportion of subjects with mRS ≤ 1 vs. the proportion with mRS>1
c. The proportion of subjects with mRS ≤2 vs. the proportion with mRS>2
d. The proportion of subjects with mRS ≤3 vs. the proportion with mRS>3
e. The proportion of subjects with mRS ≤4 vs. the proportion with mRS>4

This means that regardless of how one chooses to dichotomize the mRS scale, the ratio of the odds of an NA-1-treated subject's being in the higher functioning category of the dichotomy to a control subject's odds, will remain the same over the entire span of the mRS scale. Thus, there is no advantage to estimating ORs singly for any of the above dichotomies. If the PO assumption holds, none of them will be significantly different from the common odds ratio. This is analogous to the proportional hazard assumption of the Cox regression model, which posits a constant ratio of treatment vs. control hazard rates at every time point in a study.

Note that the model tests and estimates associated with endpoints "b" and "c" are the more commonly used (and less efficient) mRS dichotomies that represent "good" vs. "poor" functioning. Thus, in addition to providing a statistically powerful test for a treatment vs. placebo shift across all the mRS scores, the POM subsumes the more

common dichotomous mRS analyses that efficacy decisions in stroke trials are often based on (Agresti 2007; Nunn 2016).[13, 14]

The results of the PO assumption tests, the common odds ratio estimate (with Wald 95% C.I.s) and corresponding Wald test statistics will be summarized in a table. Actual proportions in each category of the collapsed mRS scale with corresponding stacked bar charts will also be presented.

In the event that the test of the PO assumption, needed to proceed with the key secondary efficacy analysis described, determines that the assumption is invalid, this analysis will not be conducted and remaining secondary endpoints to be tested as listed in Section 7.2.2 to 7.2.5 will still be considered to be protected (overall trial alpha still controlled).

7.2.2 National Institutes of Health Stroke Scale

The NIHSS is a standardized neurological examination score, with scores ranging from 0 to 42, with higher scores indicating increasing severity. The scale includes measures of level of consciousness, extra ocular movements, motor and sensory tests, coordination, language and speech evaluations. The NIHSS will be administered at Baseline, Post-EVT (2 Hours), 24 and 48 Hours, Day 5 or discharge, and Days 30 and 90.

The NIHSS scores will be dichotomized into 0-2 (indicating a good neurological outcome) versus >2 (indicating otherwise). The proportion of subjects achieving a good neurological outcome at Day 90 or the last rating in NA-1 versus placebo control subjects will be compared using the same logistic regression model as in the primary efficacy analysis. Results will be summarized and tabulated.

7.2.3 Barthel Index

The BI is an index of functional independence with scores ranging from 0 to 100, with higher scores indicating greater independence in activities of daily living and mobility. The BI will be scored at Baseline (pre-morbid) and on Days 30 and 90. Note that the original Barthel Index was a scale from 0-20, but this study will use the modified version. The modified Barthel index simply multiplies the original scale by 5.

The BI scores will be dichotomized at 0-90 (indicating otherwise) versus 95-100 (indicating independent functioning with activities of daily living). The proportion of subjects with independent functioning with activities of daily living at Day 90 in NA-1 versus placebo control subjects will be compared using the same logistic regression model as in the primary efficacy analysis. Results will be summarized and tabulated.

7.2.4 Mortality

Mortality will be assessed at Day 5 or discharge and at Days 30 and 90. Mortality rates, defined as the number of deaths observed divided by the number of subjects observed over the 90-day study period between NA-1 and placebo control subjects, will be analyzed by the same logistic regression model as in the primary efficacy. Subjects with missing outcomes will be treated as deaths for this analysis. The odds ratio associated with the treatment effect, adjusted for the stratification and covariates as described for the primary endpoint analysis, will be presented with a 95% confidence interval and the associated Wald test.

7.2.5 Proportion of subjects with Day 90 mRS ≤ 1

The Day 90 mRS score will be dichotomized at mRS≤1 (indicating freedom from disability) vs. mRS>1 (indicating otherwise). The proportion of subjects with freedom from dependence/disability based on this dichotomy on Day 90 in NA-1 versus placebo control subjects will be compared using the same logistic regression model as in the primary efficacy analysis. Results will be summarized and tabulated.

7.3 Tertiary Efficacy Outcomes Analyses

Summary statistics for each tertiary efficacy endpoint will be tabulated by treatment group. The tertiary analyses will be considered exploratory. The tertiary efficacy endpoints include the:

- Health-related quality of life, as measured by the EQ-5D-5L at Day 90 or the last rating.
- Volume of acute stroke as measured by a) DWI and b) FLAIR at 24 Hour follow-up. If NCCT was done instead of MRI, Volume of stroke will be derived from hypodense areas.

- Cognitive outcomes, as measured by the 15-item Boston Naming Test (BNT15), Sunnybrook Hemispatial Neglect Procedure (SNAP) and the Montreal Cognitive Assessment (MoCA).
- The proportion of subjects experiencing a favorable functional outcome 30 days post-randomization, defined as 0 to 2 on the mRS.

7.3.1 EQ-5D-5L

The EQ-5D-5L is a generic instrument for describing and valuing health. It is based on a descriptive system that defines health in terms of five dimensions: Mobility, Self-Care, Usual Activities, Pain/Discomfort, and Anxiety/Depression. Each dimension has five response categories corresponding to no problems, slight, moderate, severe and extreme problems. The instrument is designed for self-completion, and respondents rate their overall health on the day of the interview on a 0-100 hash-marked, vertical visual analogue scale (EQ-VAS). The EQ-5D-5L will be completed at Day 30 and Day 90.

For the EQ-5D-5L, the difference between NA-1 and placebo control subjects in the distribution of the EQ-VAS score at Day 90 will be summarized descriptively and modeled as a continuous variable. An analysis of covariance (ANCOVA) model will be fit to the EQ-VAS endpoint with the NA-1/placebo treatment indicator variable and the 7 stratification variables and covariates used in the primary and secondary efficacy analyses. ANCOVA results will be summarized in a table.

7.3.2 Volume of Stroke

All subjects will undergo a follow-up brain MRI [including a minimum of axial DWI, gradient-echo (GRE), FLAIR] at 24 ± 12 hours from the time of randomization. The 24-hour MR is considered a standard of care imaging procedure; if MR is unavailable, then NCCT is allowed. The 24 hour MR (and where MR is unavailable, CT) will be used to assess 24 Hour infarct volume. Infarct volume determinations will not be conducted until after database lock.

The total volume of new a) DWI and b) FLAIR lesions in NA-1 versus placebo control subjects will be calculated from the 24-hour imaging. Where MR is not available, infarct volumes will be determined from the 24-hour CT scan. The plan for combining CT and MRI data will be detailed in the imaging adjudication charter. Total volume

will be assessed using a linear regression using a cubic root transformation if needed. An ANCOVA will be fit and summarized as per the EQ-VAS model.

7.3.3 Cognitive Outcomes

These will be explored using the BNT15, SNAP and the MoCA scores. The BNT15 is a widely used neuropsychological assessment tool to measure confrontational word retrieval in individuals with aphasia or other language disturbance caused by various neurological disorders. The SNAP is a short bedside battery for visuoconstructive hemispatial neglect. The MoCA has been found to be a feasible global cognitive screening tool in stroke trials.

The BNT15 (short form), SNAP and MoCA will be administered at Day 90. Assessments will only be conducted if the tool/scale is available in the local country language. If the assessment is not conducted for language reasons the outcome will not be measured for those subjects.

BNTP (scale=0-15) and SNAP total scores (scale=0-100) will be analyzed and summarized as continuous endpoints using the basic ANCOVA models described in the two previous sections. As is standard practice, the MoCA scores (0-30) will be dichotomized at 26 and analyzed in the same type of logistic regression model as has been described for the primary endpoint. MoCA scores ≥ 26 are considered to indicate normal cognition (Nasreddine, 2005, Smith, 2007).[15, 16] Results will be summarized and tabulated as in the primary efficacy analysis.

7.3.4 mRS at 30 Days

The proportion of subjects experiencing a favorable functional outcome 30 days post-randomization, defined as 0 to 2 on the mRS, will be analyzed in the same type of logistic regression model as the primary endpoint. Results will be summarized and tabulated as in the primary efficacy analysis.

7.4 Exploratory Analyses

In addition to the primary and secondary analyses adjusting for age, sex, baseline NIHSS score, baseline ASPECTS, intravenous alteplase, declared first device, occlusion location and site, exploratory subgroup analyses will be conducted to

determine whether any of these factors can modify the effect of the NA-1 vs. placebo treatments. Sub-group analyses will be performed on the primary outcome and include forest plots to display effect sizes by sub-group[6].

Dichotomous sub-groups of interest include the following:

1. Age > 80 years of age
2. Sex (men vs. women)
3. Treatment with intravenous alteplase (yes/no)
4. Treatment by first declared thrombectomy device (Stent vs. Aspiration)
5. Subjects whose time from onset of stroke symptoms to NA-1 (or placebo) treatment was ≤ 6 hrs vs. > 6 hours.
6. Severe stroke defined as NIHSS > 20 vs. NIHSS <= 20.
7. Outcomes by recognized ethnic and racial groups.
8. Outcomes by degree of reperfusion (TICI ≥ 2b vs. TICI <2b)
9. General anesthesia vs. no general anesthesia
10. Baseline occlusion location (MCA vs. ICA)
11. Subjects whose time from onset of stroke symptoms to NA-1 (or placebo) treatment was ≤ 4 hrs vs. > 4 hours.
12. Subjects weighing between 105-120 kg.
13. ASPECTS score of 5-7 vs. 8-10

Additional sub-groups may be examined, but those specified above are of prior clinical interest.

Effect sizes will be estimated as subgroup-specific odds ratios (±95% CIs) as follows. Separate logistic regression models for the primary endpoint, with treatment group and the stratification variables and covariates used in the minimization algorithm as predictors, will be fit to each of the subject subgroups (e.g., a model will be fit to males

and a second model will be fit to females). The estimated (NA-1/placebo) odds ratios, with 95% confidence intervals will be the NA-1 effect size estimates for each of the subgroups (e.g., for males and for females) and will be displayed in the forest plots.

An additional exploratory analysis will consider the time from administration of tPA to infusion start and its effect on the primary efficacy outcome. This will be assessed by performing the logistic regression for the primary efficacy outcome, with an additional term added for the time interval between tPA administration and start time of infusion. This time may be positive or negative, depending on whether tPA administration or infusion occurred first, although it is expected that most subjects will receive tPA before drug infusion. The time should be calculated as "start time of drug infusion" minus "time of tPA administration".

In addition to the subgroup analyses described above, the analyses of the primary and secondary endpoints will be repeated with additional terms for period (e.g., before/after 100-subject IDMC; before/after 300-subject IDMC; before/after 600-subject IDMC) and period by treatment interaction. Simple effects within period and the interaction of treatment with period will be viewed to assess the homogeneity of the study data across periods before and after the various IDMC meetings. Also, the percentage of patients with functional independence at 90 days as defined by a 90 day mRS of 0-2 will be plotted against the time from stroke symptom onset to the time of study drug administration for each of NA-1 and placebo.

7.5 Pharmacokinetic Analyses

PK samples will be collected at baseline and at multiple time points after the complete dose was administered from up to 100 subjects enrolled at a subset of sites in Canada and the US.

Actual sampling time-points will be recorded and used for PK calculations. If data permit, the following PK parameters for NA-1 will be calculated at the end of the study by standard noncompartmental methods for all subjects with PK samples:

- AUC0–t: Area under the concentration-time curve from time zero to time of last measurable concentration

- AUC0–inf: Area under the concentration-time curve from time zero to infinity
- Cmax: Maximum plasma concentration observed after dosing
- Tmax: Time to occurrence of Cmax
- t½: Terminal elimination half-life

Samples with no detectable NA-1 will be excluded from analysis (placebo).

Descriptive statistics will be calculated for NA-1 plasma concentrations and for all PK parameters (AUC_{0-t}, $AUC_{0-\infty}$, C_{max}, T_{max}, $t_{½}$).

A further analysis of the effect of administration of thrombolytic therapy (alteplase) on the pharmacokinetics of NA-1 will also be performed.

Depending on the ability to obtain an adequate number of PK samples, the SAP may be amended (providing that no unblinding of treatment allocation has taken place) to optimize the PK analysis, including the possibility of popPK analyses in the event that only sporadic samples are obtained from subjects due to operational limitations.

Table 2. Summary of Inferential Efficacy Analyses

Endpoint Type	Endpoint	Scale	Population	Statistical Model
Primary	Day-90 mRS	Binary (≤2 vs. >2)	ITT	logit(binary mRS) modeled as a function of treatment, baseline ASPECTS, occlusion location, age, sex, baseline NIHSS, alteplase*thromb_app, study site
	Day-90 mRS	Binary (≤2 vs. >2)	PP	logit(binary mRS) modeled as a function of treatment, baseline ASPECTS, occlusion location, age, sex, baseline NIHSS, alteplase*thromb_app, study site
	Day-90 mRS	Binary (≤2 vs. >2)	ITT, re-randomization	logit(binary mRS) modeled as a function of treatment, baseline ASPECTS, occlusion location, age, sex, baseline NIHSS, alteplase*thromb_app, study site; run model on 5,000 re-randomized ITT populations
	Day-90 mRS	Binary (≤2 vs. >2)	PP	logit(binary mRS) modeled as a function of treatment, baseline ASPECTS, occlusion location, age, sex, baseline NIHSS, alteplase*(actual first endovascular approach), study site
Secondary	Day-90 mRS	Ordinal (0-4,5+6)	ITT	mRS cumulative logits modeled as a function of treatment, baseline ASPECTS, occlusion location, age, sex, baseline NIHSS, alteplase*thromb_app, study site
	Day-90 NIHSS	Binary (≤2 vs. >2)	ITT	logit(binary NIHSS) modeled as a function of treatment, baseline ASPECTS, occlusion location, age, sex, baseline NIHSS, alteplase*thromb_app, study site
	Day-90 Barthel	Binary (<95 vs.≥95)	ITT	logit(binary Barthel Index) modeled as a function of treatment, baseline ASPECTS, occlusion location, age, sex, baseline NIHSS, alteplase*thromb_app, study site
	Day-90 Mortality	Binary day-90 death status	ITT	logit(binary death status) modeled as a function of treatment, baseline ASPECT, occlusion location, age, sex, baseline NIHSS, alteplase*thromb_app, study site
	Day-90 mRS	mRS Binary (≤1 vs. >1)	ITT	logit(binary mRS) modeled as a function of treatment, baseline ASPECTS, occlusion location, age, sex, baseline NIHSS, alteplase*thromb_app, study site
Tertiary	EQ-VAS	0-100	ITT	EQ-VAS modeled as functions of treatment, baseline ASPECTS, occlusion location, age, sex, baseline NIHSS, alteplase*thromb_app, study site
	Stroke volume	continuous	ITT	Stroke volume (or cubic root transformation of stroke volume) modeled as a function of treatment, baseline ASPECTS, occlusion location, age, sex, baseline NIHSS, alteplase*thromb_app, study site
	BNT15	0-15	ITT	BNT15 Score modeled as a function of treatment, baseline ASPECTS, occlusion location, age, sex, baseline NIHSS, alteplase*thromb_app, study site
	SNAP	0-100	ITT	Total SNAP Score modeled as a function of treatment, baseline ASPECTS, occlusion location, age, sex, baseline NIHSS, alteplase*thromb_app, study site
	MoCA	Binary (<26 vs. ≥26)	ITT	logit(binary MoCA) modeled as a function of treatment, baseline ASPECTS, occlusion location, age, sex, baseline NIHSS, alteplase*thromb_app, study site
	Day-30 mRS	Binary (≤2 vs. >2)	ITT	logit(binary mRS) modeled as a function of treatment, baseline ASPECTS, occlusion location, age, sex, baseline NIHSS, alteplase*thromb_app, study site
Exploratory	Day-90 mRS	Binary (≤2 vs. >2)	ITT	Separate logistic regression models in which the logit(binary mRS)is modeled as a function of treatment only, by each of the subgroups to be considered in the Subgroup forest plots
	Day-90 mRS	Binary (≤2 vs. >2)	ITT	logit(binary mRS) modeled as a function of treatment, baseline ASPECTS, occlusion location, age, sex, baseline NIHSS, alteplase*thromb_app, study site, period, and treatment by period interaction

Endpoint Type	Endpoint	Scale	Population	Statistical Model
	All secondary endpoints	As listed above	ITT	Modeled as described above as a function of treatment, baseline ASPECTS, occlusion location, age, sex, baseline NIHSS, alteplase*thromb_app, study site, period, and treatment by period interaction
	Day-90 mRS	Binary (≤2 vs. >2)	ITT	logit(binary mRS) modeled as a function of treatment, baseline ASPECTS, occlusion location, age, sex, baseline NIHSS, alteplase*thromb_app, study site, and time between tPA administration and infusion start

8.0 SAFETY ANALYSES

The safety population will consist of all subjects who received any dose of study drug. The main analyses will be frequency of SAEs and 90-day mortality. It is expected that the safety population and the ITT population will be near-identical.

8.1 Adverse Events

Additional analyses will consider the frequency of AEs and discontinuations due to AEs.

AEs will be collected until Day 30 and SAEs will be collected until Day 90 or the final contact. AEs will be summarized by presenting, for each treatment group, the number and percentage of subjects having at least one AE, having an AE in each body system and preferred term, by severity and relatedness to study medication. The frequencies and incidences of AEs occurring in subjects in the drug and placebo control groups will be summarized within treatment group by the Medical Dictionary for Regulatory Activities (MedDRA) System Organ Class (SOC). The frequencies and incidences of discontinuations due to AEs occurring in subjects in the NA-1 and placebo control groups will be summarized within treatment group.

If a given subject had more than one AE mapped to the same preferred term, then that subject will be counted only once within that preferred term.

When reporting TEAEs by maximum severity, if a given subject had more than one AE mapped to the same preferred term, then that AE will be counted once according to the maximal level of severity (Severe, Moderate, Mild).

When reporting TEAEs by relationship to study treatment, if a given subject had more than one AE mapped to the same preferred term, then that AE will be counted once according to the highest level of relatedness (Related, Probably, Possibly, Unrelated).

The following summaries (tables) of AEs and TEAEs will be provided by number (percentage) of subjects for each treatment group:

- All TEAEs by MedDRA preferred term

- All TEAEs by MedDRA SOC and by preferred term
- TEAEs (with a start date 0-30 days) by MedDRA SOC and by preferred term
- Serious TEAEs (with start date 0-90 days) by MedDRA SOC and by preferred term
- All TEAEs resulting in death by MedDRA SOC and by preferred term
- Event rate (%) for Serious TEAEs over the 90-day study period between NA-1 and placebo control subjects will be compared by a logistic regression model similar to that of the primary analysis.
- All TEAEs occurring in at least 5% of subjects in either treatment arm, by MedDRA SOC and by preferred term.
- All TEAEs by maximum severity (Severe, Moderate, Mild) by MedDRA SOC and by preferred term. Missing severity grades will be assumed as 'severe'.
- All TEAEs by relationship to study treatment (Related, Probably, Possibly, Unrelated) by MedDRA SOC and by preferred term. Missing relationships will be assumed as 'related'.
- All TEAEs resulting in discontinuation of treatment, by MedDRA SOC and by preferred term
- TEAEs (onset 0-120 minutes post end of study drug infusion and of special interest as defined in Appendix 1) by MedDRA SOC and by preferred term
- The following listings of AE occurrences will be provided:
  - All AEs by MedDRA SOC and by preferred term
  - All SAEs by MedDRA SOC and by preferred term
  - All AEs leading to death by MedDRA SOC and by preferred term
  - All AEs related to study drug by MedDRA SOC and by preferred term
  - All deaths by treatment group

8.2 Vital Signs

Vital signs will be taken in the supine position at Baseline (pre-dose), at the completion of drug infusion, and at 24 Hours, 48 Hours and Day 5/discharge. Vital signs including temperature, systolic and diastolic blood pressure, heart rate, and weight will be

reported by assessment time. Absolute values and changes for vital signs from pre-dose to Day 5 or discharge will be documented. The maximum deviation of BP from Baseline between drug and placebo control groups (systolic and diastolic) to 24 Hours will each be analyzed using ANCOVA, including factors for treatment group, the use of general anesthesia and treatment with intravenous alteplase, three factors that may affect blood pressure. Results will be tabulated and listed by treatment group and timepoint.

8.3 Laboratory and 12-Lead Electrocardiogram Results

CBC, electrolytes, INR, aPTT, serum creatinine and serum glucose will be reported at 24 ± 12 hours. A 12-lead ECG will be performed at Baseline or, if the treating physician deems that an ECG will impede the subject's access to care, within 6 hours of randomization, and at 24 ± 12 hours.

Absolute values for laboratory results and generalized 12-lead ECG results (i.e., normal, sinus bradycardia, etc.) will be summarized descriptively. Inferential statistics (ie, p-values or CI) will not be provided for these data.

8.4 Prior and Concomitant Medications

Prior medications are defined as those taken within three days of treatment initiation. Concomitant medications (conmeds) will be collected from the time of randomization to the Day 30 Visit/contact. Prior and concomitant medications will be listed verbatim within treatment group.

8.5 Independent Data Monitoring Committee

An Independent Data Monitoring Committee (IDMC) will perform periodic safety reviews of the clinical data. The reviews will occur after 100, 300, and 600 subjects have reached their 90-day study visit. The Independent Statistical Group will generate safety reports, which will include cumulative summary statistics; subject status in the study (e.g., number completed Day 90 visits); baseline characteristics; safety data, including adverse events (AEs) and serious adverse events (SAEs) by AE code, severity, and relatedness to the study medication and discontinuations due to AEs. Two versions of these safety reports will be created – an open (blinded) report to be distributed to the Trial Executive Committee and the IDMC, and a closed (unblinded)

report to be distributed only to the IDMC. The closed reports will be forwarded to the Trial Executive Committee following database lock and unblinding at the end of the study.

The IDMC will be unblinded to safety data to ensure a detailed analysis of safety. To ensure minimization of operational bias and confidentiality of the safety data, the IDMC reports will be analyzed by an unblinded group (the "Independent Statistical Center") that is independent of the sponsor and the blinded project team who will implement the trial. Firewalls will be maintained between these two groups. No unblinded data reports will be seen or discussed by or with the blinded team during the trial. See the IDMC Charter (separate document) for additional details.

The unblinded Independent Statistical Group (ISG) will be sequestered from the Project Team, steering committee and investigators. The ISG will produce the IDMC Safety Reports as well as the Interim Analysis of Efficacy and provide them to the IDMC members. The reports to the IDMC will be provided prior to the meeting. A list of planned tables listings and figures to be included in the safety and efficacy reports are provided in Appendix A. Additional details of the interim analysis are provided in Section 3.0 of this document.

The Statistical Group is responsible to:

- Prepare Tables, Figures and Listings for the IDMC to review
- Apply the treatment codes to the data to produce the partially unblinded reports by treatment group (Group A vs Group B).
- Perform a quality check of the results
- Forward the agreed-upon Tables, Figures and Listings to the IDMC

The IDMC Project Administrator, also a member of the unblinded Statistical Group, will handle most communication between the IDMC and the Project Team, including the forwarding of the unblinded reports to the IDMC members and preparation of the Open and Closed Session meeting minutes. The IDMC Independent Reporting Statistician, also a member of the Statistical Group, also attends the Open and Closed Sessions of the IDMC meetings and answers any questions from the IDMC regarding the reports.

In contrast, the Project Statistician is on the blinded Project Team and will not produce, review or have access to unblinded aggregate reports for the IDMC during the study. The Project Statistician's group will produce the Final Study Report after final database lock and unblinding of the trial.

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Appendix 1: Listing of AEs of Special Interest

Table A-1: AEs Related to Angioedema (by PT) based on SMQ

• Allergic oedema • Angioedema • Circumoral oedema • Conjunctival oedema • Corneal oedema • Epiglottic oedema • Eye oedema • Eye swelling • Eyelid oedema • Face oedema • Gingival oedema • Gingival swelling • Idiopathic angioedema • Idiopathic urticaria • Laryngeal oedema • Laryngotracheal oedema • Limbal swelling • Lip oedema • Lip swelling • Mouth swelling • Oedema mouth • Oropharyngeal oedema • Oropharyngeal swelling • Palatal oedema • Palatal swelling • Periorbital oedema • Pharyngeal oedema • Scleral oedema • Swelling face • Swollen tongue • Tongue oedema • Tracheal oedema	• Auricular swelling • Breast oedema • Breast swelling • Choking • Choking sensation • Drug hypersensitivity • Ear swelling • Endotracheal intubation • Generalised oedema • Hypersensitivity • Laryngeal obstruction • Localised oedema • Nasal oedema • Nipple oedema • Nipple swelling • Oedema • Oedema mucosal • Oedema peripheral • Orbital oedema • Peripheral swelling • Reversible airways obstruction • Skin oedema • Skin swelling • Stridor • Suffocation feeling • Throat tightness • Tracheal obstruction • Tracheostomy • Upper airway obstruction • Urticaria • Wheezing

Table A-2: AEs related to Hypotension (by PT) based on MedDRA Terms

• Blood pressure abnormal • Blood pressure decreased • Blood pressure diastolic abnormal • Blood pressure diastolic decreased • Blood pressure difference of extremities • Blood pressure fluctuation • Blood pressure immeasurable • Blood pressure inadequately controlled • Blood pressure orthostatic abnormal	• Blood pressure orthostatic decreased • Blood pressure systolic abnormal • Blood pressure systolic decreased • Blood pressure systolic inspiratory decreased • Labile blood pressure • Hypotension • Diastolic hypotension • Hypotensive transfusion reaction • Orthostatic Hypotension

Table A-3: AEs related to Anaphylactic reaction and Anaphylactic shock (by PT) based on SMQs

Anaphylactic reaction	Anaphylactic shock
• Anaphylactic reaction • Anaphylactic shock • Anaphylactic transfusion reaction • Anaphylactoid reaction • Anaphylactoid shock • Circulatory collapse • Distributive shock • Kounis syndrome • Shock • Shock symptom	• Acute kidney injury • Acute respiratory failure • Asthma • Bronchial oedema • Bronchospasm • Cardio-respiratory distress • Dyspnoea • Erythema • Eye pruritus • Flushing • Generalised erythema • Hyperventilation • Hypoperfusion • Injection site urticaria • Jugular vein distension • Laryngospasm • Myocardial depression • Nodular rash • Ocular hyperaemia • Oropharyngeal spasm • Organ failure • Prerenal failure • Propofol infusion syndrome • Pruritus • Pruritus allergic • Pruritus generalised • Rash • Rash erythematous • Rash generalised • Rash pruritic • Renal failure • Respiratory arrest • Respiratory distress • Respiratory failure • Sensation of foreign body • Tachypnoea • Cardiac arrest • Cardio-respiratory arrest • Cardiovascular insufficiency