A Multicentre, Randomized, Double-blinded, Placebo-controlled, Parallel Group, Single-dose Design to Determine the Efficacy and Safety of Nerinetide in Participants with Acute Ischemic Stroke Undergoing Endovascular Thrombectomy Excluding Thrombolysis (ESCAPE-NEXT Trial)

PROTOCOL NA-1-009

Version 6.0
DATE: 01 June 2022

Compound: Nerinetide (NA-1)

Coordinating Centre
University of Calgary
Foothills Medical Centre
1403 29th Street NW
Calgary, Alberta, Canada T2N 2T9

Sponsor
NoNO Inc.
479A Wellington Street West
Toronto, Ontario, Canada M5V 1E7

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Signatures of Approval

Protocol No: NA-1-009

Study Title: A Multicentre, Randomized, Double-blinded, Placebo-controlled, Parallel Group, Single-dose Design to Determine the Efficacy and Safety of Nerinetide in Participants with Acute Ischemic Stroke Undergoing Endovascular Thrombectomy Excluding Thrombolysis (ESCAPE-NEXT Trial)

My signature below confirms that I have read and approved this protocol, and assures that this clinical study will be conducted according to all requirements of this protocol, the Declaration of Helsinki, International Conference for Harmonization Guideline for Good Clinical Practice (ICH-GCP), the Tri-Council Policy Statement (2), where applicable.

Personal Protected Data

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Table of Contents

Tables and Figures

1 PROTOCOL SUMMARY

1.1 Synopsis

Title	A Multicentre, Randomized, Double-blinded, Placebo-controlled, Parallel Group, Single-dose Design to Determine the Efficacy and Safety of Nerinetide in Participants with Acute Ischemic Stroke Undergoing Endovascular Thrombectomy Excluding Thrombolysis
Trial Code	ESCAPE-NEXT (NA-1-009)
Trial Design	This study is a Phase 3, randomized, multicentre, blinded, placebo-controlled, parallel group, single-dose design with a single interim analysis for safety and efficacy. Because AIS is a medical emergency, the trial is designed to enable the administration of standard-of-care treatments without delay in order to save the life of the person concerned, restore good health or alleviate suffering. At total of up to 850 male and female participants aged 18 years and older harboring an acute ischemic stroke who are selected for endovascular revascularization without intravenous or intra-arterial thrombolytic therapy will be given a single, 2.6 mg/kg (up to a maximum dose of 270 mg) intravenous dose of nerinetide or placebo. Randomization will be stratified by time from stroke onset to randomization ≤4.5 hours (yes/no) and done with stochastic minimization to balance baseline factors within strata. Outcomes of the main trial will be evaluated throughout a 90 day observation period. Participants will be contacted by telemedicine or telephone at 1-Year by individuals blinded to the outcome of the main trial. Two database locks and corresponding reports are planned for this trial. The first report will be based on the completion of Day 90 visits for the main trial. The second report will be following the completion of the 1-Year follow up for the analytic sub-trial.
Trial Objectives for Main Trial	The primary objective is to determine the efficacy of the neuroprotectant, nerinetide in: • Reducing global disability in participants with acute ischemic stroke (AIS) The secondary objectives are to determine the efficacy of nerinetide in: 1) Reducing mortality rate 2) Reducing worsening of stroke* 3) Reducing functional dependence 4) Improving neurological outcome * Worsening of stroke is defined as (A) progression, or hemorrhagic

	transformation of the index stroke, as documented by medical imaging that is (a) life-threatening requiring intervention and/or (b) results in increased disability as gauged by a ≥4 point increase from lowest NIHSS during hospitalization or (B) results in death from the index stroke. The tertiary objectives are to determine the efficacy of nerinetide in: • Decreasing infarct volume • Improving activities of daily living • Reducing dependency or death • Improving excellent functional outcome • Improving health related quality of life The safety objectives are to determine the safety in participants with acute ischemic strokes of a single 2.6 mg/kg dose (up to a maximum dose of 270 mg) of intravenous nerinetide, based on serious adverse events (SAEs) and 90-day mortality.
Efficacy Outcomes for Main Trial	The primary outcome is: • The proportion of participants with independent functioning on the modified Rankin Scale (mRS), as defined by a score of 0-2, at Day 90. The secondary outcomes include: 1) Mortality rate, as defined by event rate (%) for mortality over the 90-day study period. 2) Proportion of participants exhibiting a worsening of their index stroke. Worsening of stroke is defined as (A) progression, or hemorrhagic transformation of the index stroke, as documented by medical imaging that is (a) life-threatening requiring intervention and/or (b) results in increased disability as gauged by a ≥4 point increase from lowest NIHSS during hospitalization or (B) results in death from the index stroke. 3) A shift of one or more categories to reduced functional dependence analyzed across the whole distribution of outcomes on the mRS at Day 90 post randomization. 4) Proportion of participants with good neurological outcome, as defined by a score of 0-2 on the NIHSS at Day 90 post randomization. Tertiary outcomes include: • Volume of stroke as measured by MRI or CT brain imaging (MRI preferred).

	• Proportion of participants with functional independence in activities of daily living, as defined by a score of ≥ 95 on the Barthel Index (BI) at Day 90 post randomization. • Proportion of participants with reduced moderate or severe disability or death, as defined by a score of 4-6 on the mRS at Day 90 post randomization. • Proportion of participants with excellent functional outcome, as defined by a score of 0-1 on the mRS at Day 90 post randomization. • Health-related quality of life, as measured by the EQ-5D-5L at Day 90.
Trial Objectives for Analytic Sub-Trial at 1-Year Follow-up	There will be an analytic 1-Year follow-up sub-trial investigating the long-term effects of nerinetide treatment. The primary objective is to determine the efficacy of the neuroprotectant, nerinetide at 1-Year post randomization in: • Reducing global disability in participants with acute ischemic stroke (AIS). The secondary objectives are to determine the efficacy of nerinetide in: • Reducing mortality rate • Improving activities of daily living • Improving health related quality of life
Efficacy Outcomes for 1-Year Follow-up	The primary outcome of the 1-Year follow-up is: • The proportion of participants with independent functioning on the modified Rankin Scale (mRS), as defined by a score of 0-2 at 1-Year post randomization. The secondary outcomes include: • A reduction in mortality rate, as defined by event rate (%) for mortality over the 1-Year study follow-up period. • The proportion of participants with independent function on activities of daily living defined on the Barthel Index (BI) with a score of ≥ 95 at 1-Year post randomization. • Health-related quality of life, as measured by the EQ-5D-5L at 1-Year post randomization.
Safety Outcomes for Main Trial	Safety outcomes include: • Serious adverse events (SAEs) to Day 90. • 90-day mortality.

	Additional Safety outcomes include: • Adverse events (AEs) to Day 30. • Discontinuations due to AEs. • Baseline and Day 2 post-dose study drug laboratory tests. • Baseline and post-dose (to Day 2) study drug vital signs.
Number of Participants	Up to 850 male and female participants harboring AIS and who are selected for endovascular revascularization without intravenous or intra-arterial thrombolytic therapy will be enrolled.
Inclusion/ Exclusion Criteria	Inclusion Criteria 1) Acute ischemic stroke (AIS) selected for emergency endovascular treatment. 2) Age 18 years or greater. 3) Onset (last-known-well) time to randomization time within 12 hours. 4) Disabling stroke defined as a baseline National Institutes of Health Stroke Score (NIHSS) a. NIHSS > 5 for internal carotid artery (ICA) and M1-middle cerebral artery (MCA) occlusion or b. NIHSS > 10 for M2-MCA occlusion. 5) Confirmed symptomatic intracranial occlusion at one or more of the following locations: Intracranial carotid I/T/L, M1 or M2 segment MCA. Tandem extracranial carotid and intracranial occlusions are permitted. 6) Pre-stroke (24 hours prior to stroke onset) independent functional status in activities of daily living with modified Barthel Index (BI) ≥ 95. Patient must be living without requiring nursing care. 7) Qualifying imaging performed less than 2 hours prior to randomization. 8) Consent process completed as per national laws and regulation and the applicable ethics committee requirements. Exclusion Criteria 1) Treated with a tissue plasminogen activator (e.g., alteplase or tenecteplase) within 24 hours before randomization. 2) Determination by the treating physician, based on current treatment guidelines and medical evidence, that treatment with a plasminogen activator is indicated. 3) Large core of established infarction defined as ASPECTS 0-4.

	4) Absent or poor collateral circulation on qualifying imaging (e.g., Collateral score of 0 or 1). 5) Any intracranial hemorrhage on the qualifying imaging. 6) Planned use of an endovascular device not having approval or clearance by the relevant regulatory authority. 7) Endovascular thrombectomy procedure is completed as defined by the presence of TICI 2c/3 reperfusion or completion of groin / arterial closure. 8) Clinical history, past imaging or clinical judgment suggesting that the intracranial occlusion is chronic or there is suspected intracranial dissection such that there is a predicted lack of success with endovascular intervention. 9) Estimated or known weight > 120 kg (264 lbs). 10) Pregnancy/Lactation; female, with positive urine or serum beta human chorionic gonadotropin (β-hCG) test, or breastfeeding. 11) Known prior receipt of nerinetide for any reason, including prior enrolment in this ESCAPE-NEXT trial. 12) Severe known renal impairment defined as requiring renal replacement therapy (hemo- or peritoneal dialysis). 13) Severe or fatal comorbid illness that will prevent improvement or follow up. 14) Inability to complete follow-up treatment to Day 90. 15) Participation in another clinical trial investigating a drug, medical device, or a medical procedure in the 30 days preceding trial inclusion.
Countries	Global, multicentre trial
Treatment	Nerinetide 2.6 mg/kg (up to a maximum dose of 270 mg or matching placebo volume) will be administered as a single 10±1minute intravenous infusion using an infusion pump starting after randomization.
Consent	**Initial Informed Consent** Participants or their legally authorized representative will be required to sign a statement of informed consent that meets the requirements of applicable national laws and regulation and the ethics committee. See Appendix 10.6.1 for additional country specific details. **Regained Capacity Consent** If the original consent process involved anyone other than the participant, and if required by local standards, consent will be sought for the

	remaining procedures from the participant once they are deemed to have regained capacity. Note: Electronic consent tools may be used for initial and regained capacity consent, as permitted under national laws and regulations and the applicable Independent Review Boards/Ethics Committee.
Randomization Method	Treatment will be assigned using 1:1 randomization (nerinetide:placebo) with a stratification based on time from stroke onset to randomization of less than or equal to 4.5 hours (yes/no) and a randomized minimization algorithm to minimize the contribution of imbalances in baseline factors (age, sex, baseline NIHSS score, baseline ASPECT score, occlusion location, time from qualifying imaging to randomization, and site).
Duration of Treatment	Participants will receive a single 10-minute infusion of study drug. This trial consists of one 90-day study period for each participant. Participants will be hospitalized for care after their acute stroke according to the current standard of care. 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). For the purpose of the analytic 1-Year sub-trial, participants will be contacted by telemedicine or telephone. The end of the study is defined as the date of the last contact of the last participant in the trial at the 1-Year follow up.
Laboratory Tests	If the participant is female and is of childbearing potential, a pregnancy test (urine or serum point-of-care pregnancy test) must be completed and a negative test result obtained prior to inclusion in the trial. In order to support the assessment of safety, baseline (pre-dose) and post-dose (Day 2) hematology, electrolytes and chemistry laboratory results will be reported and analysed.
Assessment of Efficacy and Power	The primary estimand will be the adjusted unconditional population difference in the mRS response (i.e., mRS score of 0-2) proportions between treatment conditions (nerinetide vs. placebo) in the target patient population at Day 90. Deaths occurring over the Day 90 period will be considered as non-responses. Assuming a 50% overall responder rate for the placebo group population (as observed in the ESCAPE-NA1 trial), there will be approximately 91.3%power to detect an 11.4% absolute effect difference between response rate (proportion of responders, with Day 90 mRS in the range 0 to 2 with nerinetide and placebo, at alpha level 0.25 one-sided (0.05 2-sided), using the planned sample size of 850 evaluable subjects, randomized 1:1, per group [EaST v6.5, 2022].

	The interim analysis is planned to take place at 60% information (primary endpoint), i.e., when approximately 510 of the target 850 patients have reached their primary endpoint assessment. The cumulative alpha spent at the interim analysis is 0.004 and final analysis 0.025, one-sided (0.05 2-sided); the stopping boundaries on the Z scale are 2.668 (interim) and 1.981 (final) and on the p-value scale 0.004 (interim) and 0.021 (final), all on the assumption that the interim is conducted at 60% information.
Statistical Assumptions	The primary and secondary efficacy endpoint analyses will be conducted on the intent-to-treat (ITT) population, defined as all randomized participants, regardless of treatment actually received. The primary analysis will be repeated on the Per Protocol (PP) population. An ITT analysis will also be conducted for the secondary endpoints, with participant grouped according to the randomized (intended) treatment. The pivotal primary analysis for the primary estimand model will be a logistic regression model with fixed effects including the treatment group, the stratification covariate of time from stroke onset to randomization ≤ 4.5 hours (yes/no) and the randomized minimization factors (age, sex, baseline NIHSS score, occlusion location, time from qualifying imaging to randomization, baseline ASPECT score, sex, and pooled site), and an interaction term of treatment by time from stroke onset to randomization. If the interaction term is not significant at the level of 0.05 it will be removed from the model. The analysis for the secondary estimands with binary endpoints will be based on the ITT population following the same methods as the primary (logistic regression based, with the odds ratios along with the 95% CI reported in addition to the primary Ge et al, 2011 method results) and secondary (two sample proportion test) analyses of the primary estimand. Mortality analysis will be additionally supported using time-to-death survival function analysis, both unadjusted shown using Kaplan-Meier analysis and adjusted via Cox proportional hazards regression. For the secondary estimand of the "mRS shift analysis", the first step in the analysis will be an analysis with mRS score 5 and 6 combined, using a proportional odds model to derive the common odds of improvement ("shift") along the mRS scale. It will be adjusted for the same covariates as the primary analysis. The mRS shift analysis will only be conducted as part of the fixed sequence testing sequence provided that the proportional odds assumption is found to be valid on testing. If it is found to be invalid, the remaining secondary endpoints will be analyzed in the fixed testing sequence specified without the mRS shift analysis; i.e. it will be removed from the fixed sequence testing sequence. Adjustment will include the same variables as the specified in the adjusted logistic regression model for the primary estimand.

	The secondary outcome of NIHSS scores at Day 90 will be dichotomized into 0-2 (indicating a good neurological outcome) versus >2 (indicating otherwise). The proportion of participant achieving a good neurological outcome at Day 90 in nerinetide versus placebo control participants will be compared using the same approach as the primary analysis, both adjusted and unadjusted. The total lesion volume of new strokes on MRI or CT brain imaging in the nerinetide versus placebo control participants will be assessed using an unadjusted two-tailed Student's t-test and supported bya linear regression that includes the stratification and minimization variables. Secondary outcomes will be assessed in a pre-defined hierarchical order. The tertiary outcomes comprising proportions of responders will be analyzed similarly to the primary outcome or will be assessed descriptively. Three separate efficacy analysis timepoints are planned for this trial. The first analysis will be at the interim analysis planned at 60% information on the primary endpoint. The second analysis timepoint will be based on the completion of Day 90 visits for the main trial. The third analysis will be following the completion of the 1-Year follow up. For the safety analysis, the frequency of SAEs, SAEs resulting in death, AEs and discontinuations due to AEs will be summarized.
Independent Data Monitoring Committee	An Independent Data Monitoring Committee (IDMC) will monitor patient safety and scientific integrity during the trial. The interim analysis for efficacy during the trial will also be assessed by the IDMC. The interim efficacy analysis will be performed after approximately 510 participants have complete the Day 90 follow-up, at 60% information on the primary endpoint.
Bioanalytical Sampling	Plasma samples from up to 100 participants in North America (Canada and the US) will be collected for potential pharmacokinetic assessment.

Table 1-1: Schedule of Activities- Main Trial

Visit/Contact	V1	V2	V3	V4	V5	V6
Day	**Day 1 Baseline**	**Day 1 Post-EVT**	**Day 2/3**	**Day 6[1] or discharge**	**Day 30[2]**	**Day 90[2]**
Window		**(~2 h)**	**(18-56 h)**		**(±5 d)**	**(-21 to +7d)**
Informed consent	X					
Regained capacity informed consent[3]			X	X	X	X
History and physical examination	X					
Weight[4]	X					
Vital Signs (BP, HR, Temperature)[5]	X	X	X			
Randomization/ Study drug administration	X					
Mortality		X	X	X	X	X
NIHSS	X	X	X	X	X	X
mRS[6]	X			X	X	X
Barthel Index	X				X	X
EQ-5D-5L						X
Qualifying Imaging	X					
Endovascular Procedure	X					
MRI/NCCT head[7]			X			
Laboratory Assessments	X[8]		X			
Pregnancy test[9]	X					
Pharmacokinetic samples[10]	X					
AE	Collected to Day 30					
SAE	Collected to Day 90					
Prior medications	X					
Concomitant medications	Collected to Day 6 or discharge					

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 a 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. 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.
5. Vital signs (BP, HR only) will be recorded immediately before and after completion of the study drug infusion, temperature will be collected at baseline only if standard of care.
6. Historical (pre-stroke) mRS score can be collected at any time.
7. MRI head may be supplanted by an NCCT head if MR is unavailable or contraindicated.
8. Blood should be drawn at baseline, but results are not required prior to randomization. Results from primary hospital (within 8 hours) are accepted.
9. If the participant 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.
10. PK samples will be collected from up to 100 participants: pre-dose and at 10, 20, 30 and 60 min after the start of study drug administration.

d = days; h = hours

Table 1-2: Schedule of Activities: 1-Year Follow Up Telemedicine or Telephone Contact

Contact	**V7** **1-Year Follow up**
Day	**Day 365**
Window	**(±30 d)**
Mortality	X
EQ-5D-5L	X
mRS	X
Barthel Index	X

1.2 Coordinating Centre and Sponsor

Coordinating Centre	Personal Protected Data
Overall Coordinating Investigator:	
Co-Coordinating Investigator	
Sponsor:	
Sponsor Medical Oversight	
Imaging Adjudication	
Pharmacokinetic Analysis	

2 INTRODUCTION

2.1 Background

Stroke is a leading cause of mortality and neurological disability worldwide[1-3]. When blood flow to the brain is interrupted during a stroke, some brain cells die immediately, while others remain at risk for death. These damaged cells make up the ischemic penumbra and can linger in a compromised state for periods varying from minutes to several hours[4-6]. Given that there is a critical time, a "therapeutic window", which may vary from minutes to a few hours in which cerebral ischemia can be reversed or mitigated, stroke should be treated as a medical emergency and treatment should commence without delay in order to save the life of the person concerned, restore good health and alleviate suffering.

Brain tissue is rapidly and irretrievably lost as stroke progresses[8] and early intervention is critical to improve stroke outcome. Alteplase, recombinant tissue plasminogen activator, is the only approved pharmacological treatment for acute ischemic stroke (AIS) and must be administered within 3-4.5 hours of symptom onset, and only in those patients for which the possibility of hemorrhagic stroke was excluded. According to the 2017 claims data only 10% of all ischemic strokes in the USA are treated with alteplase.

Endovascular thrombectomy (EVT) is being used with or without alteplase to retrieve blood clots in AIS caused by large vessel occlusion (LVO)[9]. Even with EVT, only about 10% patients return to normal after their AIS[9], and only approximately half reach functional independence[10]. Therefore, although reperfusion therapies improve stroke prognosis, there remains a significant unmet medical need. Such a need would be fulfilled by a neuroprotective therapy – one that enhances the brain's resilience to ischemia. However, at present, no approved neuroprotective pharmacotherapy exists.

Nerinetide (NA-1) is a first in class neuroprotectant that is designed to address the major unmet medical need for treatments that reduce the functional disability produced by acute stroke. It reduces the vulnerability of ischemic brain tissue to hypoperfusion by targeting neurotoxic pathways that lead to ischemic neuronal death. Nerinetide is intended, alone or in combination with available therapies, to treat acute stroke, a serious and life-threatening disease.

For this reason, nerinetide is being developed as a drug for use in emergency situations aimed at reducing global disability in patients with acute ischemic stroke. Nerinetide 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 nerinetide. The ability to identify patients with salvageable brain using the criteria used in the ESCAPE trial[11] and the ESCAPE-NA1 trial[12] provides an opportunity to target patients who may have the greatest benefit from neuroprotection, and to enhance further the impact of reperfusion therapies. The preclinical and clinical data support this notion.

A detailed description of the chemistry, pharmacology, efficacy, and safety of nerinetide is provided in the Investigator's Brochure.

2.1.1 Nerinetide (NA-1): A Post-synaptic Density 95 Inhibitor

Nerinetide (NA-1) is a first in class neuroprotectant that is designed to address the major unmet medical need for treatments that reduce the functional disability produced by acute stroke. It reduces the vulnerability of ischemic brain tissue to hypoperfusion by targeting neurotoxic pathways that lead to ischemic neuronal death. Nerinetide is intended, alone or in combination with available therapies, to treat acute stroke, a serious and life-threatening disease.

Nerinetide (NA-1) is a novel synthetic peptide composed of two parts: a 9 amino acid active substance that binds to post-synaptic density protein 95 (PSD-95), and an 11 amino acid sequence that allows delivery across the blood brain barrier and cell membranes into the neuronal target cells. Nerinetide is designed to target post-synaptic density protein-95 (PSD-95), which binds both the N-methyl-D-aspartate receptors (NMDARs) and neuronal nitric oxide synthases (nNOS) at excitatory synapses to form the NMDAR/PSD-95/nNOS complex that efficiently translates NMDAR overactivation to NO production during cerebral ischemia. Nerinetide inhibits the protein-protein interaction between PSD-95 and the GluN2B subunits of NMDARs, as well as the interactions between nNOS and PSD-95[13]. This inhibition uncouples nNOS from NMDAR activity in order to prevent or limit the onset of neuronal excitotoxicity that is associated with AIS and other disorders in which glutamatergic mechanisms play a pathophysiological role. Nerinetide has no effect on other known NMDAR functions, but results in decreases in downstream neurotoxic signaling (i.e., NO production). Figure 1 summarizes the mechanism of inhibition of nitric oxide (NO) by nerinetide (NA-1).

Based on pharmacokinetic studies, the plasma half-life of nerinetide at doses in the therapeutic range is in the 6-15 minute range.

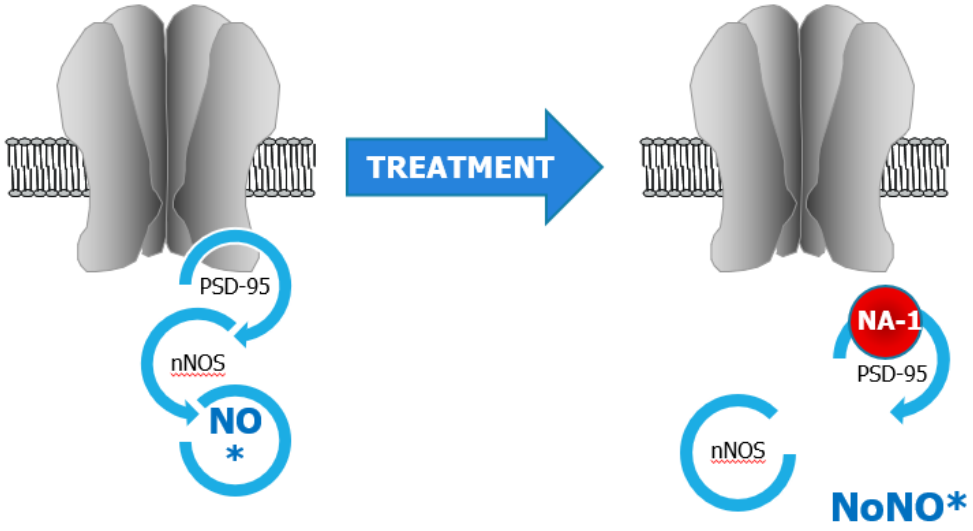

Figure 1: Inhibition of NO Production by Nerinetide (NA-1) via Perturbation of NMDA Receptor PSD-95 Interactions

2.1.2 Impact of Alteplase on Nerinetide

Nerinetide is intended to be used as a standard of care stroke therapy. The only currently approved pharmacological agent for treating AIS is alteplase, a thrombolytic agent. Nerinetide has amino-acid sequences known to be cleaved by plasmin, a serine protease generated from clot-bound plasminogen by tissue-plasminogen activators such as alteplase[14]. Nerinetide does not have any intrinsic fibrinolytic activity and does not affect the activity of thrombolytics such as alteplase or tenecteplase[15] but the converse is different. Plasmin, a serine protease, is activated by thrombolytic drugs to dissolve fibrin blood clots and, although plasmin itself has a very short half life in human blood, its effects persist for several hours[16]. Plasmin has a similar substrate specificity to trypsin[17], which is predicted to cleave nerinetide after residues 3, 4, 5, 6, 7, 9, 11 and 12 from the N-terminus (https://web.expasy.org/peptide_cutter/). Similar cleavage products were observed after incubating nerinetide with plasmin *in vitro*[18]. Incubating nerinetide with alteplase in rat or human plasma reduced the nerinetide content in both. Concurrent administration of nerinetide and alteplase to rats caused a significant lowering of nerinetide levels[18].

2.1.3 Thrombectomy As a Stroke Treatment

Medical devices may be used with or without alteplase to retrieve blood clots in large cerebral arteries that cause severe brain ischemia ("endovascular thrombectomy")[9]. The evidence that endovascular therapy (EVT) is effective in improving neurological outcome is strongest in patients who have the combination of an LVO as well as direct or indirect imaging evidence of salvageable brain (an ischemic penumbra) at the time of treatment initiation. Current generation devices are intended for patients whose AIS is caused by a LVO, and produce higher rates of reperfusion than alteplase in such appropriately selected patients. However, even with EVT, only about 10% patients return to normal as defined by modified Rankin Score (mRS) = 0 after their

AIS[9], and only approximately half become functionally independent[10]. In other words, about half of patients who receive EVT still remain functionally dependent or die from their stroke. Moreover, although still the participant of intense study in ongoing randomized trials, clinical data available to date suggest there is no significant difference in outcomes between patients who were treated with EVT only as compared with those who received combined thrombolysis with alteplase and EVT[19-21].

Although reperfusion therapies such as alteplase and/or EVT improve stroke prognosis, a significant need remains to reduce the overall number of AIS patients who have poor outcomes. As such, stroke remains a serious condition, with an unmet medical need.

2.1.4 Treatment of Stroke with Nerinetide in Non-Human Primates

To test whether nerinetide is beneficial when administered later in the setting of a prolonged temporary middle cerebral artery occlusion (tMCAO), 24 cynomolgus macaques received a 10-minute infusion of nerinetide or placebo three hours after the onset of a 3.5 hour tMCAO. There were no mortalities. Final imaging and neurological assessments were conducted at 14 days. Nerinetide treated animals exhibited significant reductions in infarct volumes as compared with placebo as evaluated on MRI (T2-weighted MRI: at 48 hours: p=0.006; DWI MRI at 48 hours: p=0.004; T2-weighted MRI at 14 Days: p=0.003).

Animals treated with nerinetide exhibited improved non-human primate stroke score (NHPSS) scores throughout the 14-day observation period days [p=0.004, two-way repeated measures analysis of variance] and trended to better performance in the six-well and the valley staircase tasks[22]. There were no statistically significant differences in any of the physiological parameters (including MAP) at any of the measured time points for the nerinetide versus placebo treated animals.

More detailed information on these and other non-human primate studies are provided in the Investigator's Brochure.

2.1.5 Previous Clinical Trials

Three clinical trials with nerinetide have been completed to date.

The results of a Phase 1 trial conducted in healthy volunteers indicate that nerinetide is well tolerated when administered in doses ranging between 0.02 and 2.60 mg/kg and a dose of 2.6 mg/kg was selected for further clinical trials. No serious adverse events (SAEs) or discontinuations due to adverse events were reported in the trial.

In the Phase 2 ENACT clinical trial using a dose of 2.60 mg/kg in patients undergoing endovascular repair of brain aneurysms, both unruptured and ruptured, the data suggest a treatment effect of nerinetide on the procedurally – induced strokes. The treatment effect was most evident when evaluating lesion counts using DWI or FLAIR imaging, and also in exploratory analyses when evaluating lesion volume in the mITT population. The treatment effect was most pronounced in participants who suffered from a ruptured brain aneurysm, in whom infarct numbers and infarct volumes were reduced. Exploratory analyses suggested that stroke volumes were also reduced when analyses accounted for delayed strokes, or for the non-normality of the data. There were three deaths during this trial, two in the placebo group and one in the

nerinetide group. The SAEs leading to death were all severe and unrelated to study drug. There were no other discontinuations due to adverse events. Overall, nerinetide (NA-1) 2.60 mg/kg was well-tolerated and no safety concerns were identified in any of the patient groups in the trial.

The selection of the single IV dose of 2.60 mg/kg is based on the safety and tolerability profile of nerinetide observed in the Phase 1 and 2 clinical trials.

In the ESCAPE-NA1 trial, treatment with single 2.6 mg/kg IV dose of nerinetide did not achieve the primary endpoint of the trial in all participants with ischemic stroke due to large vessel occlusion and who were selected for EVT, with and without intravenous alteplase. Among participants who were not treated with alteplase, a treatment effect was observed. Specifically, there was a benefit in the nerinetide group on the proportion of participants achieving an mRS 0-2 at 90 days (59.4% for nerinetide participants vs. 49.8% for placebo participants) (Odds Ratio = 1.657; 95% CI 1.055, 2.603; p = 0.028). There was also a reduction in mortality rate in the participants receiving nerinetide with an absolute reduction in mortality rate of 7.5% (relative difference of 39.7%; p = 0.041, Fisher's Exact Test) without an increase in severe disability (i.e., mRS 4 or 5). Other measures of function, including the NIHSS and BI trended in the same direction, in favor of nerinetide. Lastly, treatment with nerinetide resulted in a significant reduction in median infarct volumes in the nerinetide group (p = 0.048).

The results of the safety analysis from the ESCAPE-NA1 trial indicate that nerinetide was well tolerated when given as a single IV dose of 2.6 mg/kg with most adverse events occurring with a similar frequency in the drug and placebo groups. The only exception to that was an increase in serous hypotension immediately (within 2 hours) following the administration of nerinetide (6 SAEs in nerinetide vs. 0 in placebo). These cases were reported resolved within 2 days. There were no other differences in other important safety outcomes observed. When nerinetide is administered without alteplase there were fewer deaths and a fewer number of neurological serious adverse events (including stroke in evolution, ischemic stroke and hemorrhagic transformation). When nerinetide is administered with alteplase there were no differences in important safety outcomes observed between the nerinetide and placebo groups.

2.2 Study Rationale

The rationale for the ESCAPE-NEXT trial is based on promising results from the recently completed ESCAPE-NA1 trial[12] and to confirm the findings that nerinetide may improve functional independence, reduce mortality, and reduce infarction volumes in participants with AIS who are selected for EVT and who are not treated with thrombolytics.

ESCAPE-NA1 was a randomized, multicentre, blinded, placebo-controlled, parallel group, single-dose trial conducted at 48 sites in the USA, Canada, Ireland, the UK, Sweden, Germany, Republic of Korea, and Australia. While a treatment benefit of nerinetide for the primary outcome in the trial population was not observed, a large absolute benefit of treatment with nerinetide over placebo was observed in participants not treated with alteplase. In the subgroup of participants that did not receive alteplase, the improvement in functional outcome was accompanied by reduced mortality and these clinical effects were mirrored by the imaging biomarker of reduced infarct volumes in the nerinetide-treated group. The effectiveness of nerinetide on improving functional independence, mortality, and infarction volumes in the no-alteplase stratum was not seen in the stratum treated with alteplase. This is consistent with the hypothesis that nerinetide

was cleaved and inactivated as a result of the administration of alteplase. The data from ESCAPE-NA1 are also consistent with a significant body of preclinical studies in rodents and primates showing that nerinetide reduces infarct burdens and improves functional outcomes in experimental animals. The effectiveness of nerinetide in participants who did not receive prior alteplase underlies the rationale for selecting such participants for the present study. This rationale is discussed further in Section 4.3.

There is a compelling need to develop neuroprotectants in order to increase the proportion of patients who may benefit from EVT. These agents could improve the outcomes of patients and render more patients with AIS into candidates for endovascular or pharmacological recanalization treatment. The rapid progression of irreversible brain injury in most acute strokes implies a short window of clinical efficacy of any treatment, including nerinetide.

2.3 Benefit/Risk Assessment

More detailed information about the chemistry, pharmacology, efficacy, safety and expected benefits and risks of nerinetide is provided in the Investigator's Brochure.

2.3.1 Risk Assessment

Based on the clinical data available for nerinetide to date, the major possible risk for the proposed use is:

- Higher rate of (transient) hypotension due to a transient elevation of blood histamine
- Effect modification by prior use of alteplase

2.3.2 Benefit Assessment

The following is a list of possible benefits to the trial participants in ESCAPE-NEXT:

- improved functional outcome (mRS 0-2)
- reduced stroke mortality
- improved good neurologic outcome (NIHSS 0-2)
- reduced chance of stroke worsening
- improved functional independence (BI>95)
- contribution to the process of developing new therapies in an area of unmet medical need

Based on the clinical data available for nerinetide to date, treatment with nerinetide may slow the progression of ischemic brain damage, providing more time during which endovascular thrombectomy may be of benefit to the patient. This is of even greater relevance during the global COVID-19 pandemic for the following reasons:

a) There is a necessity to protect hospital staff, resulting in additional hospital protocols involving sanitations and personal protective equipment that minimize the exposure of the clinical stroke team to a potentially COVID-19 positive stroke patient (there is no time for COVID-19 testing to be completed).

b) There is a necessity to protect the patient, resulting in additional hospital protocols involving distancing him/her from hospital staff and sanitizing equipment (e.g., CT scanner) used on other patients.

These necessities slow down emergency stroke care workflows, potentially causing undue delays in the emergency stroke care of all AIS patients, not just those who may have been exposed to COVID-19. Thus, the possibility that nerinetide, by slowing the progression of ischemic brain damage, mitigates such delays may be of direct benefit to treated patients. Further details related to COVID-19 considerations are provided in Section 13.1.

2.3.3 Overall Benefit: Risk Conclusion

The potential risks identified in association with nerinetide are justified by the anticipated benefits that may be afforded to participants with acute ischemic stroke.

Overall, nerinetide administered as a single intravenous dose was well tolerated at doses up to and including 2.6 mg/kg, and no safety concerns have been identified in any of the patient groups in the clinical trials.

3 TRIAL OBJECTIVES

3.1 Objectives

Table 3-1: Objectives and Endpoints

Objectives	Endpoints
Primary	
Reducing global disability in participants with acute ischemic stroke (AIS).	The proportion of participants with independent functioning on the modified Rankin Scale (mRS), as defined by a score of 0-2 at Day 90.
Secondary	
Reducing mortality rate.	Proportion of participant mortality over the 90-day study period.
Reducing worsening of stroke	Proportion of participants with a worsening of stroke over the 90-day study period.
Reducing functional dependence.	A shift of one or more categories to reduced functional dependence analyzed across the whole distribution of outcomes on the mRS at Day 90.
Improving neurological outcome.	Proportion of participants with a score of 0-2 on the NIHSS at Day 90.
Tertiary/Exploratory	
Decreasing infarct volume.	Volume of stroke as measured by MRI or CT brain imaging (MRI preferred).
Improving activities of daily living.	Proportion of participants with a score of ≥ 95 on the Barthel Index (BI) at Day 90.
Reducing dependency or death.	Proportion of participants with a score of 4-6 on the mRS at Day 90.
Improving excellent functional outcome.	Proportion of participants with a score of 0-1 on the mRS at Day 90.
Improving health related quality of life.	Health-related quality of life, as measured by the EQ-5D-5L at Day 90.
Safety	
To determine the safety based on serious adverse events (SAEs).	Proportion of participants with serious adverse events to Day 90.
90-day mortality.	Proportion of participants alive at 90-day.

1-Year Follow Up

Objectives	Endpoints
Reducing global disability in participants with acute ischemic stroke (AIS).	Proportion of participants with independent functioning on the modified Rankin Score (mRS) score of 0-2 at 1-Year.
Reducing mortality rate.	Proportion of participant mortality at 1-Year.
Improving activities of daily living.	Proportion of participants with a score of ≥ 95 on the Barthel Index (BI) at 1-Year.
Improving health related quality of life.	Health-related quality of life, as measured by the EQ-5D-5L at 1-Year.

3.1.1 Primary Objective

The primary objective is to determine the efficacy of the neuroprotectant, nerinetide in reducing global disability in participants with acute ischemic stroke (AIS).

3.1.2 Secondary Objectives

The secondary objectives are to determine the efficacy of nerinetide in:

1) Reducing mortality rate
2) Reducing worsening of stroke*
3) Reducing functional dependence
4) Improving neurological outcome

* Worsening of stroke is defined as (A) progression, or hemorrhagic transformation, of the index stroke as documented by medical imaging that is (a) life-threatening requiring intervention and/or (b) results in increased disability as gauged by a ≥4 point increase from lowest NIHSS during hospitalization or (B) results in death from the index stroke.

3.1.3 Tertiary Objectives

The tertiary objectives are to determine the efficacy of nerinetide in:

- Decreasing infarct volume
- Improving activities of daily living
- Reducing dependency or death
- Improving excellent functional outcome. Improving health related quality of life

3.1.4 Safety Objectives

The 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 infusion of nerinetide to participant with acute stroke on SAEs and 90-day mortality.

3.1.5 1-Year Follow Up Analytic Sub-Trial Objectives

There will be a 1-Year follow-up analytic sub-trial to support the outcomes obtained at Day 90.

The primary objective is to determine the efficacy of the neuroprotectant, nerinetide in:

- Reducing global disability in participants with acute ischemic stroke (AIS).

The secondary objectives are to determine the efficacy of nerinetide in:

- Reducing mortality rate
- Improving activities of daily living
- Improving health related quality of life

3.2 Outcomes

3.2.1 Primary Efficacy Outcome

The primary outcome is the proportion of participants with independent functioning on the modified Rankin Scale (mRS), as defined by a score of 0-2 at Day 90 post randomization. These participants are defined to be responders (See Section 9.3.1 for further details).

3.2.2 Secondary Efficacy Outcomes

Secondary outcomes include:

1) A reduction in mortality rate, as defined by event rate (proportion, expressed as a percentage) for mortality over the 90-day study period.
2) Proportion of participants with worsening of stroke over the 90-day study period.
3) A shift of one or more categories to reduced functional dependence analyzed across the whole distribution of outcomes on the mRS at Day 90 post randomization.
4) Proportion of participants with good neurological outcome, as defined by a score of 0-2 on the NIHSS at Day 90 post randomization.

3.2.3 Tertiary Outcomes

The following tertiary outcomes will be assessed descriptively:

- Volume of stroke as measured by MRI or CT brain imaging (MRI preferred).
- Proportion of participants with functional independence in activities of daily living, as defined by a score of ≥ 95 on the Barthel Index (BI) at Day 90 post randomization.
- Proportion of participants with reduced moderate or severe disability or death, as defined by a score of 4-6 on the mRS at Day 90 post randomization.
- Proportion of participants with excellent functional outcome, as defined by a score of 0-1 on the mRS at Day 90 post randomization.
- Health-related quality of life, as measured by the EQ-5D-5L at Day 90.

3.2.4 1-Year Follow Up Analytic Sub-Trial Outcomes

The primary outcome is the proportion of participants with independent functioning on the modified Rankin Scale (mRS), as defined by a score of 0-2 at 1-Year.

The secondary outcomes include:

- A reduction in mortality rate, as defined by event rate (%) for mortality over the 1-Year study period.
- The proportion of participants with independent function on activities of daily living defined on the modified Barthel Index (BI) with a score of ≥ 95 at 1-Year.
- Health-related quality of life, as measured by the EQ-5D-5L at 1-Year.

3.2.5 Safety Outcomes

The safety outcomes are the frequencies of SAEs and 90-day mortality.

4 TRIAL DESIGN

4.1 Overall Design

This study is a Phase 3, randomized, multicentre, blinded, placebo-controlled, parallel group, single-dose design with a single interim analysis for safety and efficacy. Because AIS is a medical emergency, the trial is designed to enable the administration of standard-of-care treatments without delay in order to save the life of the person concerned, restore good health or alleviate suffering.

Participants harboring an acute ischemic stroke who are selected for endovascular revascularization without intravenous or intra-arterial thrombolytic therapy will be given a single, 2.6 mg/kg (up to a maximum dose of 270 mg) intravenous dose of nerinetide or placebo. Randomization will be stratified by time from stroke onset to randomization ≤ 4.5 hours (yes/no) and done with stochastic minimization to balance baseline factors within strata. The end of the main trial is defined as the date that the last enrolled participant has completed their Day 90 visit/contact. For the purpose of an analytic follow-up sub-trial component, participants will be contacted by telemedicine or telephone at 1-Year by individuals blinded to the outcome of the main trial.

A total of up to 850 male and female participants aged 18 years and older harboring AIS and who are selected for endovascular revascularization without intravenous or intra-arterial thrombolytic therapy will be enrolled.

All participants in the main trial will be followed for 90 days (or until death if prior to 90 days). 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).

Participants will be followed at 1-Year for the analytic sub-trial for further outcome assessment by telemedicine or telephone interview conducted by individuals blinded to the outcome of the main trial. This sub-trial will be conducted to explore the independent functioning and quality of life at 1-Year.

Two database locks and corresponding reports are planned for this trial. The first report will be based on the completion of Day 90 visits for the main trial. The second report will be following the completion of the 1-Year follow up for the analytic sub-trial.

4.2 Scientific Rationale for Study Design

The rationale for the present trial is as follows:

1) The ESCAPE-NA1 trial (protocol NA-1-007) provided promising evidence that, in participants with AIS who were selected for EVT and who did not receive thrombolysis, treatment with nerinetide increases functional independence and reduced stroke mortality[12]. This was supported by a reduction in infarction volume as measured by MRI or CT scanning. Since these improvements were observed in three separate domains of outcome (functional independence, mortality, and infarction volumes), they are unlikely to be due to chance alone. The present trial is intended to explore these findings further.

2) Participants in ESCAPE-NA1 who received thrombolysis as part of their care did not benefit from subsequently being administered nerinetide. The hypothesis explaining this lack of effect is that nerinetide in participants who received alteplase was cleaved by the protease plasmin. Plasmin is produced from circulating plasminogen by alteplase and persists for hours after alteplase treatment. This hypothesis of nerinetide cleavage was supported by pharmacokinetic data from a subset of participants in ESCAPE-NA1 that showed that those who received thrombolysis had reduced plasma levels of nerinetide (approximately 60% reduction) as compared to participants who did not receive thrombolysis., Because nerinetide is a peptide drug, it was predicted to be cleaved by plasmin, though the magnitude of this effect in humans was unclear. Preclinical studies showed that plasmin cleaves nerinetide into several fragments beginning at its N-terminus. Additional preclinical studies then showed that co-administration of nerinetide with high-dose alteplase (6x the human dose) can nullify the neuroprotective effectiveness of nerinetide in a rat model of embolic middle cerebral artery occlusion (eMCAO). However, due to differences between human and rat fibrinolytic systems, the magnitude of the drug-drug interaction between alteplase and nerinetide, and its impact on the effectiveness of nerinetide in humans have not been anticipated from these animal studies. Section 4.3 provides further details regarding the justification of the target patient population for this trial as a result of these preclinical and clinical findings.
3) Based on data from preclinical studies, the Phase 1 (protocol NA-1-001) safety trial, the Phase 2 ENACT trial[23] (protocol NA-1-002) and the Phase 3 ESCAPE-NA1 trial[12] (protocol NA-1-007), nerinetide is expected to be have an acceptable safety profile.
4) There is a compelling need to develop neuroprotectants in order to increase the proportion of patients who may benefit from EVT. These agents could improve the outcomes of patients and render more patients with AIS into candidates for endovascular or pharmacological recanalization treatment.

The current trial is intended to confirm the findings in the ESCAPE-NA1 study that nerinetide may improve functional independence, reduce mortality, and reduce infarction volumes in participants with AIS who are selected for EVT and who are not treated with thrombolytics. As such, it is a Phase 3, randomized, multicentre, blinded, placebo-controlled, parallel group, single-dose design. Participants harboring an acute ischemic stroke who are selected for endovascular revascularization without intravenous or intra-arterial thrombolytic therapy will be given a single, 2.6 mg/kg (up to a maximum dose of 270 mg) intravenous dose of nerinetide or placebo. Randomization will be stratified by time from stroke onset to randomization ≤ 4.5 hours (yes/no) and done with stochastic minimization to balance baseline factors within strata.

The current trial is timely, in that it is evaluating an intervention that may mitigate the detrimental effects of slowdowns in emergency stroke care workflows caused by COVID-19 (see Section 2.3.2). For example, if reperfusion is delayed by 30 minutes, the probability of a good outcome is reduced by 10.6%[24] and perhaps up to 26%[25]. Treatment with nerinetide in patients who are not treated with thrombolysis improved good outcome by a similar degree in ESCAPE-NA1[12].

The main differences between this trial (ESCAPE-NEXT) and ESCAPE-NA1 are that:

1) ESCAPE-NEXT focuses on participants who do not receive thrombolysis, in order to confirm the results in that patient population observed in ESCAPE-NA1
2) The secondary outcomes in ESCAPE-NEXT focus on domains of clinical benefit other than functional outcome, namely mortality, rates of worsening of stroke and neurological function.
3) ESCAPE-NEXT has an analytic 1-Year follow-up sub-trial investigating the long-term effects of nerinetide treatment.

4.3 Justification for the Target Study Population

Nerinetide is being developed as a drug for use in emergency situations aimed at reducing global disability in patients with acute ischemic stroke. Nerinetide is intended to enhance the outcome of patients who may benefit from EVT and add to the existing standard of care stroke treatments.

Since the only approved pharmacological therapy for acute ischemic stroke is recombinant tissue plasminogen activator (rtPA; alteplase), NoNO has conducted several pre-clinical studies to evaluate and understand the drug-drug interaction between nerinetide and thrombolytic agents such as alteplase or tenecteplase. Nerinetide does not have fibrinolytic activity of its own, nor does it interfere with the activity of alteplase or tenecteplase in humans. It has a plasma half-life of about 5-10 minutes after which it is redistributed to extravascular tissues. Because nerinetide is a peptide, it is cleaved by proteases. Nerinetide is shown to be cleaved by plasmin, a serine protease generated from the maturation of circulating plasminogen by tissue-plasminogen activators such as alteplase and tenecteplase. In preclinical studies in-vitro, nerinetide levels in rat plasma were reduced by alteplase in a concentration-dependent manner, and the effect of a concentration of 22.5 µg/ml (intended to mimic the concentration of alteplase in humans during a clinical infusion) on lowering nerinetide levels was similar in magnitude between rat and human plasma. When given in-vivo, the co-administration of nerinetide with the human dose of alteplase (0.9 mg/Kg) resulted in a non- significant reduction of the Cmax and AUC of nerinetide. However, at six times the human dose (5.4mg/Kg) alteplase caused a significant lowering of the mean Cmax and AUC of nerinetide (49.5% and 44%, respectively). Details of these findings are summarized in the Investigator's Brochure.

NoNO's previous Phase 3 trial (ESCAPE-NA-1) accounted for the existing standard of care stroke treatments, namely the use of alteplase, and the use of different thrombectomy devices, in accordance with institutional standards. In anticipation of a possible drug-drug interaction between nerinetide and alteplase, the trial employed stratification based on the use of alteplase and the thrombectomy device, and a minimization approach randomization in a 1:1 ratio for nerinetide vs. placebo. The ESCAPE-NA1 study provided significant evidence supporting an interaction between the use of alteplase and the efficacy of nerinetide. In the no-alteplase stratum, nerinetide was associated with improved outcomes, and in the alteplase stratum there was no observed benefit with the absolute risk difference slightly (non-significantly) favoring placebo. The biological plausibility of treatment effect modification by alteplase was supported by findings from PK sampling from subjects in the trial showing reductions in peak plasma nerinetide levels in subjects that received alteplase, as well as by the preclinical data that demonstrated the cleavage of nerinetide by alteplase in rat and human plasma and in-vivo in rats. Details of these findings are summarized in the Investigator's Brochure.

Due to the clear and non-arbitrary findings of a drug-drug interaction between thrombolytics and nerinetide, it was decided that the current ESCAPE-NEXT study would focus its design on participants who are not treated with alteplase. Current stroke treatment guidelines[26] recommend the use of thrombolysis only up to 4.5 hours after stroke onset, whereas the enrollment window for ESCAPE-NEXT is up to 12 hours. Additionally, a large proportion of stroke patients who present within the 4.5 hour window of alteplase do not qualify for thrombolysis due to various reasons[27-29]. Therefore, it is anticipated that the ESCAPE-NEXT trial will not interfere with clinical decisions made by the treating physicians, while addressing a substantial and important portion of patients with AIS who are selected for EVT.

Because of this important distinction between subjects in the alteplase stratum vs the no-alteplase stratum in the previous ESCAPE-NA-1 study, the current study (ESCAPE-NEXT) is designed to address a population of adults 18 years and older harboring an acute ischemic stroke who are selected for endovascular revascularization without prior treatment with intravenous or intra-arterial thrombolytic therapy.

4.4 Justification for Dose

Nerinetide 2.6 mg/kg, up to a maximum of 270 mg (or matching placebo volume) is administered as a single approximately 10-minute intravenous infusion in the upper or lower extremity using an infusion pump initiated in the CT scan suite. The 2.6 mg/kg dose was chosen for this clinical trial because of:

1) the safety profile observed in the previous Phase 1, 2 and 3 clinical trials,
2) the observed capacity of this dose of nerinetide to reduce stroke tissue damage and to improve neurological function in humans and non-human primates and
3) the capacity of this dose to reduce stroke tissue damage and improve neurological damage in human participants undergoing endovascular repair of brain aneurysms as demonstrated in the phase 2 trial.
4) the capacity of this dose to improve functional independence (mRS 0-2) as demonstrated in the Phase 3 trial

4.5 End of Study Definition

The end of the study is defined as the date of the last contact of the last participant in the trial at the 1-Year follow up.

Two database locks and corresponding reports are planned for this trial. The first report will be based on the completion of Day 90 visits for the main trial. The second report will be following the completion of the 1-Year follow up for the analytic sub-trial.

5 STUDY POPULATION

Prospective approval of protocol deviations to recruitment and enrollment criteria, also known as protocol waivers or exemptions, is not permitted. The study is designed to address a population of participants who are not deemed by the treating physician to be candidates for thrombolytic therapy according to current guidelines. The study population includes adults 18 years and older harboring an acute ischemic stroke who are selected for endovascular revascularization without prior treatment with intravenous or intra-arterial thrombolytic therapy. This population is substantially similar to that in the ESCAPE-NA1 trial but excludes patients who received or are planned to receive prior thrombolysis.

The decision to administer a thrombolytic is at the discretion of the treating physician independently of the trial. The treating physician is expected to follow standard of care guidelines, consider best medical evidence including any hypersensitivity reactions of the patient to the drug, and to use their clinical judgement in order to ensure the best care for their acute stroke patients. Patients who are deemed by the treating physician to benefit from thrombolysis, or are administered a thrombolytic prior to screening for any reason, will not be enrolled in ESCAPE- NEXT. If the patient is not selected for treatment with a thrombolytic the treating physician may consider the patient as a candidate for the trial.

5.1 Inclusion Criteria

1) Acute ischemic stroke (AIS) selected for emergency endovascular treatment.
2) Age 18 years or greater.
3) Onset (last-known-well) time to randomization time within 12 hours.
4) Disabling stroke defined as a baseline National Institutes of Health Stroke Score (NIHSS):
    a. NIHSS > 5 for internal carotid artery (ICA) and M1-middle cerebral artery (MCA) occlusion; or
    b. NIHSS > 10 for M2-MCA occlusion.
5) Confirmed symptomatic intracranial occlusion at one or more of the following locations: Intracranial carotid I/T/L, M1 or M2 segment MCA. Tandem extracranial carotid and intracranial occlusions are permitted.
6) Pre-stroke (24 hours prior to stroke onset) independent functional status in activities of daily living with modified Barthel Index (BI) ≥ 95. Patient must be living without requiring nursing care.
7) Qualifying imaging performed less than 2 hours prior to randomization.
8) Consent process completed as per national laws and regulation and the applicable ethics committee requirements.

5.2 Exclusion Criteria

1) Treated with a tissue plasminogen activator (e.g., alteplase or tenecteplase) within 24 hours before randomization

2) Determination by the treating physician, based on current treatment guidelines and medical evidence, that treatment with a plasminogen activator is indicated.
3) Large core of established infarction defined as ASPECTS 0-4.
4) Absent or poor collateral circulation on qualifying imaging (e.g., collateral score of 0 or 1).
5) Any intracranial hemorrhage on the qualifying imaging.
6) Planned use of an endovascular device not having approval or clearance by the relevant regulatory authority.
7) Endovascular thrombectomy procedure is completed as defined by the presence of TICI 2c/3 reperfusion or completion of groin / arterial closure.
8) Clinical history, past imaging or clinical judgment suggesting that the intracranial occlusion is chronic or there is suspected intracranial dissection such that there is a predicted lack of success with endovascular intervention.
9) Estimated or known weight > 120 kg (264 lbs).
10) Pregnancy/Lactation; female, with positive urine or serum beta human chorionic gonadotropin (β-hCG) test, or breastfeeding.
11) Known prior receipt of nerinetide for any reason, including prior enrolment in this ESCAPE-NEXT trial.
12) Severe known renal impairment defined as requiring renal replacement therapy (hemo- or peritoneal dialysis).
13) Severe or fatal comorbid illness that will prevent improvement or follow up.
14) Inability to complete follow-up treatment to Day 90.
15) Participation in another clinical trial investigating a drug, medical device, or a medical procedure in the 30 days preceding trial inclusion.

5.3 Lifestyle Considerations

No restrictions are required.

5.4 Screen Failures

Screen failures are defined as patients who consent to participate in the clinical trial but are not subsequently randomized to be a trial participant. The informed consent form will be maintained at the study site, but these participants will not be entered in the CRF.

5.5 Study Enrolment Process

In this acute stroke trial, participants should be randomized into the trial and receive study drug as soon as possible, following review of trial eligibility and the local informed consent process. Participants will be identified using usual standard of care screening methods at the acute stroke hospital. All participants will undergo an acute clinical assessment, blood laboratory assessment and baseline brain imaging.

If the participant remains eligible after completion of routine stroke screening, the patient will be consented (as required) and enrolled into the trial. A participant is considered randomized the moment the randomization process is completed on-line. Participants who are randomized but do not receive study drug will still be followed through the 90-day study period.

5.5.1 Imaging

The purpose of qualifying imaging is to identify a population of patients with stroke due to large vessel occlusion and to exclude patients with already malignant infarctions at baseline as those patients are predicted to lack or have small ischemic penumbras which are the targets of stroke therapy. The imaging criteria comprise a simple minimal standard set of qualifying imaging across the sites involved in the trial which are consistent with current standards of practice at the trial sites and stroke guidelines, to allow generalizability of the trial results[30, 31].

Minimum qualifying imaging is a NCCT and CTA with an imaging time from first slice NCCT to randomization time <= 120 minutes. mCTA is preferred but if it cannot be done, CTP or multimodal MR may be used instead.

Instructions for the determination of the ASPECT score and collaterals scoring on CTA are provided at www.aspectsinstroke.com.

Sites will only be selected to participate in the trial if they have established mechanisms for screening this population of participant. This includes standard of care use of imaging.

Patients will be included if they meet the following qualifying imaging criteria:

- Non-contrast CT scan or MR-DWI scan with ASPECTS > 4, AND;
- A proven anterior circulation intracranial occlusion (ICA, M1, M2) defined by CTA (preferred) or MRA;

Patients will be excluded if they meet the following imaging criteria:

- Direct or indirect evidence of poor pial collateral filling derived by any of the following:

  Direct pial collateral assessment

  1. mCTA evidence of poor pial collaterals – Tan score of 0 or 1
  2. spCTA evidence of poor pial collaterals if no mCTA is available–Tan score of 0 or 1
  3. dynamic mCTA (derived from CTP imaging acquisition) may be used if mCTA or spCTA is not available.

  Indirect pial collateral assessment

  1. If mCTA cannot be done collaterals may be assessed/inferred based upon perfusion imaging (CT perfusion or MR perfusion). As a guide if there is a match deficit (estimated core = estimated penumbra) collaterals are poor. If there is a mismatch (estimated penumbra > estimated core) AND estimated core <70 cc, we infer that pial collaterals are adequate.

  If there is discordance between mCTA and the allowable alternate modalities, mCTA criteria should be used.

5.5.2 Consent Process

Participants or their legally authorized representative will be required to sign a statement of informed consent that meets the requirements of applicable national laws and regulation and the ethics committee.

The investigator or his/her representative will explain the nature of the trial to the participant or his/her legally authorized representative and answer all questions regarding the trial.

The medical record must include a statement describing under which process consent was obtained, and the timing of the consent and regained capacity consent. The authorized person obtaining the informed consent must also sign the informed consent form (ICF).

See Appendix 10.6 for additional country specific details.

Regained Capacity Consent

If the original process involved anyone other than the participant, and if required by local standards, consent will be sought for the remaining procedures from the participant once they are deemed to have regained capacity. Site staff will make ongoing efforts until: (1) regained capacity consent is obtained from participant as soon as it is possible and reasonable, (2) death, or (3) completion of the Day 90 assessment.

In the event the participant dies prior to providing regained capacity consent, each site will have a plan to provide information about the clinical trial to the subject's LAR or family member.

Amendment of the ICF

In the event that new information is available and the ICF is amended, and if required by local Health Authorities and/or Ethics Committees, participants must be re-consented to the most current version of the ICF(s) during their participation in the trial. A copy of each ICF(s) must be provided to the participant or the participant's legally authorized representative.

Note: Electronic consent tools may be used for initial and regained capacity consent, as permitted under national laws and regulations and the applicable Independent Review Boards/Ethics Committee.

5.5.3 Physical Examinations

To support the assessment of inclusion and exclusion criteria and medical history, a stroke focused physical examination at baseline will include, at a minimum, assessments of the Neurological, Cardiovascular, Respiratory and Gastrointestinal systems. Investigators should pay special attention to clinical signs related to previous strokes.

6 STUDY INTERVENTIONS and CONCOMITANT THERAPY

6.1 Study Intervention Administration

Nerinetide was formulated at 20 mg/ml under current Good Manufacturing Practices by Confidential Business Information in 50 mM sodium phosphate buffer with 0.45% sodium chloride (NaCl), potential hydrogen (pH) 7.0. This formulation was dispensed aseptically into 20 mL single use vials with snap cap lids (13.5 mL) intended for a single-dose intravenous infusion of nerinetide.

Placebo consists of the same buffer used for nerinetide with slightly higher NaCl content to adjust for equivalence of osmolality between drug product and placebo. It is supplied in identical vials containing 13.5 mL of 50 mM sodium phosphate pH 7.0 (0.55% NaCl). After dilution into the saline bag, the drug has been demonstrated to be stable for at least 5 hours.

Formulated nerinetide and placebo will be provided to clinical trial sites in sterile, single-use individually labeled vials (serum vials) each with a unique five-digit identification number and will be stored at the clinical trial site at 2 to 8°C in a secure location with restricted access.

Table 6-1: Study Interventions

	Nerinetide	**Placebo**
Test Product Name	Nerinetide (NA-1)	Placebo
Type	Drug	Drug
Dose Formulation	Vial	Vial
Unit Dose Strength(s)	2.6 mg/kg	Placebo
Dosage Level(s)	Single	Single
Route of Administration	IV Infusion	IV Infusion
Use	Investigational	Placebo
Sourcing	Provided centrally by the Sponsor	Provided centrally by the Sponsor
Packaging and Labeling	Study Intervention will be provided in a 20 mL vial. Vials will be packaged in boxes of multiple vials labeled as required per country requirements.	Study Intervention will be provided in a 20 mL vial. Vials will be packaged in boxed of multiple vials labeled as required per country requirements.

6.2 Preparation/ Handling/ Storage/ Accountability

Only participants enrolled in the trial may receive study intervention and only authorized site staff may supply or administer study intervention. Study drug dosing will be carried out by, or under the supervision of, the investigator who is supervising the care of the participant for the planned or ongoing endovascular reperfusion procedure.

As soon as the participant is deemed eligible for the trial, the investigator (or delegate) will log into the central randomization website to receive the assigned vial number.

Dose timing starts at the time of infusion onset. Study drug is intended to be administered as soon as possible after randomization (i.e., 15 minutes).

A syringe for each individual participant's dosing will be prepared by calculating the volume to draw from the vial as follows:

- for participants weighing less than 105 kg: (2.6 mg/kg x participant weight in kg)/(20 mg/ml). This will determine the number of mL to pull up into the syringe.
- for participants weighing 105-120 kg the full volume of one vial (13.5mL, equivalent to 270 mg of nerinetide) will be used.

Estimated or actual weight can be used to calculate the study drug dosage.

The syringe containing study drug will be injected into the intravenous port of a 50 or 100 mL drip bag of 0.9% normal saline that has been labeled with the randomization number. The bag will be mixed by squeezing and inverting the bag several times. Study drug should be infused into the participant as soon as practical.

Dosing will be performed by administering the contents of the bag of study drug to the participant through an intravenous catheter inserted into a vein in the upper or lower extremity and using a standard infusion pump. Dosing will be carried out evenly over the course of 10 ± 1 minutes. The entire volume (treatment dose) of the intravenous bag must be administered.

After the dose administration, a minimum of 10 mL of saline will be administered using the infusion pump, to flush any remaining medication left within the intravenous tubing.

6.2.1 Storage and Accountability

All study drug must be stored in a secure, temperature controlled (2 to 8 °C), and monitored (manual or automated) area in accordance with the labeled storage conditions with access limited to the investigator and authorized site staff. Temperatures will be monitored using a device that can continuously monitor and record temperature readings.

The investigator is responsible for study drug accountability, reconciliation, and record maintenance (i.e., receipt, reconciliation, and final disposition records). Any expired, lost, damaged or out-of-specification study drug must be properly documented and reported to the Sponsor. Further guidance and information for the final disposition of unused study drug are provided in the Operating Guideline.

6.2.2 Disposition of Study Drug Supplies

After study drug preparation, any remaining material in the vial will be labeled as "used," and the volume removed will be documented (value in mL). Vials labeled as "used" will be retained in a separate storage container than the unused vials. Used vials will be disposed of on site, once the monitor (CRA) has completed the study drug monitoring and accountability. Unused vials will be disposed of on site or returned to the sponsor after they have been monitored by the sponsor or sponsor's representative. Destruction of unused vials at the study site will be documented and conducted in accordance with the site's policies and procedures.

6.3 Measures to Minimize Bias: Randomization and Blinding

All participants will be centrally assigned to a randomized study intervention (i.e., nerinetide or placebo) using an Interactive Web Response System (IWRS). The login information and directions for the IWRS will be provided to each site. The IWRS website will allocate the participant number and vial number.

Treatment allocation will be assigned using 1:1 randomization (nerinetide:placebo) with a stratification based on time from stroke onset to randomization of ≤4.5 hours (yes/no) and a randomized minimization algorithm to minimize the contribution of imbalances in baseline factors (age, sex, baseline NIHSS score, baseline ASPECT score, occlusion location, time from qualifying imaging to randomization, and site). Randomization will be conducted centrally, using a web-based algorithm with treatment assignment allocated by web-based real-time interaction with the site. Randomized allocation will be fully concealed by having both dynamic real-time allocation based upon random number generation and blinded by the use of visually identical appearing nerinetide and placebo vials. All vials will have a unique vial number.

The purpose of minimization on the variables is to balance these variables across treatments and within strata to ensure a reduced chance that any observed effect size of nerinetide vs. placebo is confounded by these known important prognostic variables. This randomized minimization method from Zhao et al, called the minimal sufficient balance method[32], will be used to ensure that the participants entered into the trial will be balanced between control (placebo) and active treatment (nerinetide) arms.

All participants, investigators, their clinical staff, local and central laboratories, the clinical coordinating centre, the data management group, and the sponsor staff and delegates will be blinded to the randomization codes.

6.3.1 Procedure for Breaking the Randomization Code

In case of an emergency, the investigator has the sole responsibility for determining if the unblinding of a participant's intervention assignment is warranted. Participant safety must always be the first consideration in making such a determination. If the investigator decides that unblinding is warranted, the investigator should make every effort to contact the sponsor prior to unblinding a participant's intervention assignment unless this could delay emergency treatment of the participant. If a participant's intervention assignment is unblinded, the sponsor must be notified within 24 hours after breaking the blind. The date and reason that the blind was broken must be recorded in the source documentation as applicable.

To maintain the overall quality and legitimacy of the clinical trial, code breaks should occur only in exceptional circumstances when knowledge of the actual treatment is absolutely essential for further management of the patient to ensure their safety and well-being.

In case of emergency, a rapid unblinding procedure is available to investigators. The investigator will contact the randomization provider to request unblinding of the specific participant. The randomization provider 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 is requested to maintain the blind as far as possible. The actual treatment allocation

should not be disclosed to the participant and/or other site personnel unless, in the judgement of the investigator, this information is required for the participant's safety. The actual treatment allocation must not be disclosed to study personnel on site not involved in the participant's medical care, to monitors or the sponsor.

In order to fulfill expedited regulatory reporting requirements, the Sponsor may be required to unblind the participant if the SAE meets the criteria for reporting to health authorities. The Sponsor's independent third party will initiate the request that the participant's treatment group be unblinded. In this case, the code will be broken only for the participant(s) in question. The information resulting from code-breaking (i.e., the participant's treatment allocation) will not be communicated to either the Investigator or the Sponsor.

Otherwise, randomization data will be kept strictly confidential, accessible only to authorized persons, until the time of unblinding after database lock at the time of interim analysis and at end of the study (Day 90). For the ongoing 1-Year follow up, site staff conducting the assessments will remain blinded until after the 1-Year follow up database lock.

6.4 Study Intervention Compliance

Study drug will be dispensed under the instructions of the investigator or designee and under medical supervision. The date and time of dose administered will be recorded in the source documents and in the CRF. The dose of study drug and study participant identification will be confirmed at the time of dosing by a member of the study site staff other than the person administering the study drug. The investigator may terminate study drug administration at his/her discretion.

6.5 Dose Modification

Not applicable in this single-dose trial.

6.6 Continued Access to Study Intervention after the End of the Study

Not applicable in this single-dose trial.

6.7 Treatment of Overdose

For this trial, any dose of study drug greater than 150% of the planned dose will be considered an overdose.

The sponsor does not recommend specific treatment for an overdose. In the event of an overdose, the investigator should:

1) Contact the Drug Safety Department (sae@nonoinc.ca) immediately.
2) Closely monitor the participant for any AEs/SAEs and laboratory abnormalities for at least 1 day.
3) Document the quantity administered in the CRF.

6.8 Concomitant Therapy

There are no restricted medications once a participant is enrolled in this trial. Any medication or vaccines (including over-the-counter or prescription medicines) that the participant is receiving at the time of enrollment (within 24 hours prior) or receives up to Day 6 must be recorded along with:

- Dates of administration including start and end dates, medications that were ongoing at the last contact will be updated with a stop date or confirmed as ongoing.
- Indication for use.

6.8.1 Rescue Medicine/Treatment

6.8.1.1 Early Study Drug Cessation

The intervention is the intravenous administration of 2.6 mg/kg (up to a maximum of 270 mg) of nerinetide over a 10 ± 1 minute intravenous infusion to participant undergoing endovascular mechanical thrombolysis/thrombectomy. It is expected that dosing will be completed before the endovascular procedure is completed. However, if dosing is ongoing during the endovascular procedure, dosing will not be stopped in the event of an adverse intra-procedural event deemed to be a complication of the endovascular intervention.

The investigator may terminate drug administration at his/her discretion.

6.8.1.2 Treatment of Hypotension

If hypotension (systolic < 80 mmHg; or any level of decreased BP that the physician deems to be clinically relevant) is observed in a participant, the hospital physician will be instructed, at his/her discretion, to administer any medication that they deem to be required for the participant's health and safety.

There is no specific treatment requirement related to treating hypotension that may be observed in participants with stroke who are also receiving nerinetide. Treatment of hypotension in this setting may include all or some of the following, as appropriate, and at the hospital physician's discretion.

First, the physician will determine if hypotension is symptomatic. Asymptomatic participants may be observed for spontaneous recovery.

- Treatment, if required, should include fluid resuscitation with crystalloid fluid (e.g., 0.9% saline) and/or vasopressors, if needed.
- Consider treatment with antihistamine agents (diphenhydramine 50 mg IV, ranitidine 50mg IV) and corticosteroids (e.g., Decadron™; 10 mg IV) if the reaction is severe.
- Consider using subcutaneous or intravenous epinephrine.
- If bronchospasm or laryngospasm are important additional symptoms, consider treatment with inhaled racemic epinephrine.

Specific amounts and doses of intravenous fluids or other drugs administered are left to the medical judgment of the hospital physician.

All participants will be monitored closely for these and other potential complications throughout the clinical trial. All participants will receive standard medical care as per local practice. If hypotension as defined above occurs, the hypotension and its treatment are to be recorded as an AE in the CRF.

6.9 Endovascular Intervention and Stroke Care

EVT should be conducted as per the local protocol and in compliance with the current treatment guidelines such as those published by the American Heart Association (AHA).

All acute stroke participants should receive the best standard of care according to current treatment guideline and any national guidelines (e.g., Canadian best practices guidelines for acute stroke care, European Stroke Organization, etc.). All participants are expected to be admitted to hospital as part of routine standard of care. All participants are expected to receive expert stroke unit care and then rehabilitation according to their clinical need through the full 90 days.

7 DISCONTINUATION of STUDY INTERVENTION and PARTICIPANT DISCONTINUATION/WITHDRAWAL

7.1 Discontinuation of Study Intervention

In rare instances, it may be necessary for a participant to permanently discontinue (definitive discontinuation) study intervention. If study intervention is definitively discontinued, the participant will remain in the trial and be evaluated to Day 90.

7.2 Participant Discontinuation/Withdrawal from the Trial

Participation in this clinical trial may be discontinued for any of the following reasons:

- Administrative reasons (uncooperative, noncompliant, etc.)
- Participant's decision not to participate any further
- If it is in the participant's best interest, per the qualified/principal or sub-investigator

If the participant or legally authorized representative (LAR) withdraws consent, participant data will be included in the analysis up to the date of the consent withdrawal and this withdrawal of consent will be documented in the CRF.

If the LAR has originally provided consent and the participant subsequently declines consent, this will be deemed to be a withdrawal of consent. The investigator and sponsor would continue to have access to data that have already been collected.

A participant may not withdraw use of his or her data that have already been collected, this is in alignment with the FDA guidance document "Data Retention When Participants Withdraw from FDA-Regulated Clinical Trials", which is based on the importance of avoiding selection biases that could compromise the analysis of the overall trial.

Otherwise, all randomized participants will continue to be followed according to protocol requirements and follow-up data will be included in the analysis. Criteria for removal of participants will be recorded and reported.

7.3 Lost to Follow up

A participant will be considered lost to follow-up if he or she repeatedly fails to return for scheduled visits/contacts and is unable to be contacted by the study site. The following actions must be taken if a participant fails to return to the clinic or if they cannot be contacted by phone for a required study visit:

- The site must attempt to contact the participant and reschedule the missed visit/contact as soon as possible and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain whether or not the participant wishes to and/or should continue in the trial.
- Before a participant is deemed lost to follow up, the investigator or designee must make every effort to regain contact with the participant (at a minimum 3 telephone calls/contacts). These contact attempts should be documented in the participant's study record.

- Should the participant continue to be unreachable, he/she will be considered to have withdrawn from the trial.
- Site personnel will attempt to collect the vital status of the participant within legal and ethical boundaries for all participants randomized, including those who did not get study intervention. Public sources may be searched for vital status information. If vital status is determined as deceased, this will be documented and the participant will not be considered lost to follow-up. Sponsor personnel will not be involved in any attempts to collect vital status information.

8 STUDY ASSESSMENTS and PROCEDURES

Study procedures and their timing are summarized in the Schedule of Activities. Adherence to the trial design requirements is essential and required for study conduct.

Procedures conducted as part of the participant's routine clinical management (e.g., blood count) and obtained before signing of the ICF may be utilized for baseline purposes provided the procedures met the protocol-specified criteria and were performed within the time frame defined in the Schedule of Activities.

8.1 Efficacy Assessments

Planned time points for all efficacy assessments are provided in the Schedule of Activities.

8.1.1 The Modified Rankin Scale

The primary endpoint used in this trial will be global disability, as measured by the mRS, at Day 90. The mRS is a valid and reliable clinician-reported measure of global disability that has been widely applied for evaluating recovery from stroke. It is a scale used to measure functional recovery (the degree of disability or dependence in daily activities) of people who have suffered a stroke[33, 34]. mRS scores range from 0 to 6, with 0 indicating no residual symptoms; 5 indicating bedbound, requiring constant care; and 6 indicating death.

The post dose mRS will be obtained at Day 6 (or discharge), Day 30 and Day 90 and at the 1-Year follow up. 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 (via www.healthcarepoint.com) in the use of this scale. An Electronic Clinical Outcome Assessment (eCOA) tool may be used to conduct this assessment.

8.1.2 Mortality Rate

Mortality status will be obtained at all visits during the 90-day study period and at the 1-Year follow up.

8.1.3 Worsening of Stroke

Worsening of stroke is defined as (A) progression, or hemorrhagic transformation, of the index stroke as documented by medical imaging that is (a) life-threatening requiring intervention and/or (b) results in increased disability as gauged by a ≥4 point increase from lowest NIHSS during hospitalization or (B) results in death from the index stroke.

8.1.4 Volume of Strokes

Prior to database lock at 90 Days, the total lesion volume of stroke as measured by MRI or CT brain images (MRI preferred) in nerinetide versus placebo control participants will be calculated from the Day 2/3 imaging. Where MRI is not available, infarct volumes will be determined from the Day 2/3 CT scan. The plan for combining CT and MRI data will be detailed in the Imaging Adjudication Charter.

8.1.5 The National Institutes of Health Stroke Scale

The NIHSS is a standardized neurological examination score that is a valid and reliable measure of disability and recovery after acute stroke[35]. Scores range 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), Day 2, Day 6 (or discharge), Day 30 and Day 90. The NIHSS will only be scored by those trained in the use of this scale. An eCOA tool may be used to conduct this assessment.

8.1.6 Barthel Index

The BI is an index of functional independence[36] that is a valid measure of activities of daily living when employed in stroke trials[37]. Modified BI scores range 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), Day 30 and Day 90 and at the 1-Year follow up, by those trained in the use of this scale. Note that the original Barthel Index was a scale from 0-20. The modified Barthel index simply multiplies the original scale by 5 to provide a 100-point score.

8.1.7 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[38]. Each dimension has five response categories corresponding to: no problems, slight, moderate, severe and extreme problems[39]. The version of the instrument selected for the trial is interviewer administered either in-person, or by telemedicine or by telephone. The respondents will also rate their overall health on the day of the interview on a 0–100 visual analogue scale (EQ-VAS). The EQ-5D-5L will be administered at the Day 90 and at the 1-Year follow up by those trained in the use of this scale. An eCOA tool may be used to conduct this assessment.

8.2 SAFETY ASSESSMENTS

Planned time points for all safety assessments are provided in the Schedule of Activities.

8.2.1 Vital Signs

Blood pressure and heart rate will be taken at Baseline (pre-dose), at the completion of drug infusion and Day 2. Results taken from standard of care assessment/timepoints may be used. Temperature will be taken at baseline (if available per standard of care) and Day 2. Clinically significant findings post-dose will be reported as AEs.

8.2.2 Clinical Safety Laboratory Assessments

Blood work will be taken at baseline and at Day 2 including: CBC (hemoglobin, platelets and hematocrit), electrolytes (sodium, potassium, chloride), serum creatinine and serum glucose and assessed by the local laboratory. Results from local standard of care testing may be used. A central lab will not be used. Due to the short time-period from arrival at hospital and the EVT, the results of the baseline blood work are not required prior to randomization. Baseline results from

primary hospital (within 8 hours) are accepted, if written documentation is available. Clinically significant laboratory findings from the Day 2 sample (post dose) will be reported as AEs.

8.2.3 Pregnancy Testing

If the participant is female and is of childbearing potential, a pregnancy test (urine or serum point-of-care pregnancy test) must be completed at baseline prior to inclusion into the trial.

8.3 Adverse Events (AEs), Serious Adverse Events (SAEs), and Other Safety Reporting

The definitions of an AE or SAE can be found in Appendix 3: Adverse Events: Definitions and Procedures for Recording, Evaluating, Follow-up, and Reporting.

AEs will be reported by the participant (or, when appropriate, by a healthcare provider, caregiver, surrogate, or the participant's legally authorized representative). The investigator and any qualified designees are responsible for detecting, documenting, and recording events that meet the definition of an AE or SAE and remain responsible for following up AEs that are serious, considered related to the study intervention or study procedures, or that caused the participant to discontinue the trial.

Adverse events of special interest (AESIs) include any AEs which occur within 2 hours of end of study drug infusion and which fall under the standardized MedDRA queries of "Angioedema", "Anaphylactic reaction", and "Anaphylactic shock" as well as a list of MedDRA terms relating to "Hypotension".

The method of recording, evaluating, and assessing causality of AEs, AESI and SAEs and the procedures for completing and transmitting SAE reports are provided in Appendix 3.

8.3.1 Time Period and Frequency for Collecting AE and SAE Information

All SAEs will be collected from the start of study drug administration until Day 90, in addition SAE assessed as related to study drug, or have a fatal outcome will be collected to Day 90, see the Schedule of Activities.

All AE will be collected from the start of study drug administration until Day 30 at the time points specified in the Schedule of Activities.

Medical occurrences that begin before the start of study intervention but after enrolment into the trial will be recorded on the Medical History section of the electronic case report form (CRF) not the AE section.

All SAEs will be recorded and reported to the sponsor or designee immediately, without undue delay, under no circumstances later than 24 hours following the knowledge of such an event, as indicated in Appendix 3. The investigator will submit any updated SAE data to the sponsor within 24 hours of it being available.

8.3.2 Method of Detecting AEs and SAEs

Care will be taken not to introduce bias when detecting AEs and/or SAEs. Open-ended and non-leading verbal questioning of the participant is the preferred method to inquire about AE occurrences. A consistent methodology of eliciting AEs at all participant evaluation timepoints will be used. Non-directive questions include:

- How have you felt since your last clinical visit/hospital discharge?
- Have you had any new or changed health problems since you were last here?
- Have you had any unusual or unexpected worsening of your underlying medical condition or overall health?
- Have there been any changes in the medicines you take since your last clinical visit/hospital discharge?

AE identification while the participant is admitted to the acute stroke hospital will be collected via acute stroke hospital patient records and verbal histories from the participant or LAR. For follow up visits after discharge from the acute stroke hospital the participant (or LAR if the participant is not able to respond to the questions) will be asked about the occurrence of AEs since the last contact, and if available, from records at the acute stroke hospital.

Diagnosis versus signs and symptoms for the purpose of AE reporting: if known at the time of reporting, a diagnosis should be reported rather than individual signs and symptoms (e.g., record only pneumonia rather than pyrexia, coughing, shortness of breath). However, if a constellation of signs and/or symptoms cannot be medically characterized as a single diagnosis it is acceptable to report the information that is ultimately available.

8.3.3 Follow-up of AEs and SAEs

After the initial AE/SAE report, the investigator is required to proactively follow each participant at subsequent visits/contacts. All SAEs will be followed until resolution, stabilization, the event is otherwise explained, or the participant is lost to follow-up (as defined in Section 7.3). Further information on follow-up procedures is given in Appendix 3.

AEs that were ongoing at the last contact will be updated with a stop date or confirmed as ongoing. AE collection will continue until Day 30, and SAE to Day 90 or the final contact.

Investigators are not obligated to actively seek AE or SAE after conclusion of the study participation. However, if the investigator learns of any SAE, including a death, at any time after a participant has been discharged from the study, and he/she considers the event to be reasonably related to the study intervention or study participation, the investigator must promptly notify the sponsor.

8.3.4 Regulatory Reporting Requirements for SAEs

Prompt notification by the investigator to the sponsor of a SAE is essential so that legal obligations and ethical responsibilities towards the safety of participants and the safety of a study intervention under clinical investigation are met.

The sponsor has a legal responsibility to notify both the local regulatory authority and other regulatory agencies about the safety of a study intervention under clinical investigation. The sponsor will comply with country-specific regulatory requirements relating to safety reporting to the regulatory authority, Institutional Review Boards (IRB)/Independent Ethics Committees (IEC), and investigators.

Investigator safety reports must be prepared by the sponsor for suspected unexpected serious adverse reactions (SUSAR) according to local regulatory requirements and sponsor policy and forwarded to investigators as necessary.

The Sponsor's Drug Safety Department will notify the Investigators in writing of the occurrence of any reportable SAEs. The Sponsor or delegate will be responsible for reporting SUSARs to any Central Ethics Committees in compliance with local current legislation. The Investigators will be responsible for informing their local ethics committees of any reportable SAEs as per their local requirements.

An investigator who receives an investigator safety report describing a SAE or other specific safety information (e.g., summary or listing of SAEs) from the sponsor will review and then file it along with the Investigator's Brochure and will notify the IRB/IEC, if appropriate according to local requirements.

8.3.5 Pregnancy

Details of all pregnancies in female participants and female partners of male participants will be collected after the start of study intervention and until Day 30.

If a pregnancy is reported, the investigator should inform the sponsor within 24 hours of learning of the pregnancy and should follow the procedures outlined in Section 10.4 (Appendix 4: Collection of Pregnancy Information).

Pregnancy itself is not considered an AE, but any complications during pregnancy are to be considered as AEs, and in some cases, could be considered SAEs. Spontaneous abortions, fetal death, stillbirth, and congenital anomalies reported in the baby are always considered as SAEs, and the information should be provided to the Sponsor regardless of when the SAE occurs (e.g., even after the end of the trial).

8.4 Pharmacokinetics

Blood samples of approximately 5 mL will be collected for measurement of plasma concentrations of nerinetide as specified in the Schedule of Activities. Samples will be collected from up to 100 participants enrolled in Canada and the US. A total of five 5 mL blood samples will be drawn during the Baseline Visit (V1). Time (T) =0 being the initiation of dosing of study drug or placebo. Samples should be drawn at the following timepoints:

Sample 1: post randomization & prior to start of study drug administration

Sample 2: 10 minutes from start of study drug administration (Time = within 1 min of study drug completion)

Sample 3: 20 minutes from start of study drug administration (Time = 15 to 25 min)

Sample 4: 30 minutes from start of study drug administration (Time = 26 to 45 min)

Sample 5: 60 minutes from start of study drug administration (Time = 46 to 120 min)

Sample collection method should be the least invasive to the participant and may be combined with routine blood sampling. Instructions for the collection and handling of biological samples

will be provided by the sponsor. The actual date and time (24-hour clock time) of each sample will be recorded.

Each plasma sample will be divided into 2 aliquots (1 each for primary and a back-up). The samples will be handled per guidelines/instructions provided by the sponsor. During storage, PK tubes will be kept in a freezer at -10°C or colder.

The plasma samples will be processed for pharmacokinetics analyses. Appropriate firewalls will be put in place to prevent unblinding. Results of the analysis will not be provided to the sponsor until after database lock. Genetic analyses will not be performed on these samples. Participant confidentiality will be maintained. Samples may be stored for a maximum of 15 years (or according to local regulations) following the last participant's Day 90 visit at a facility selected by the sponsor

9 STATISTICS

9.1 Sample Size Determination

The primary estimand will be the adjusted difference in the mRS response (i.e., mRS score of 0-2) proportions between treatment conditions (nerinetide vs. placebo) in the target patient population at Day 90. Participants with mRS score of 0-2 are defined to be responders. Deaths occurring over the Day 90 period will be considered as non-responses.

Primary hypothesis test and 95% confidence interval estimate for the primary estimand will be provided by the analytic method of Ge, 2011[40] discussed in FDA's most recent draft guidance Adjusting for Covariates in Randomized Clinical Trials for Drugs and Biological Products[41], adjusting for the stratification covariate of time from stroke onset to randomization ≤ 4.5 hours (yes/no) and the randomized minimization factors (age, sex, baseline NIHSS score, baseline ASPECT score, occlusion location, time from qualifying imaging to randomization and pooled site), and an interaction term of treatment by time from stroke onset to randomization. If the interaction term is not significant at the level of 0.05, it will be removed from the model.

Up to 850 male and female participants aged 18 or older harboring AIS and who are selected for endovascular revascularization without intravenous or intra-arterial thrombolytic therapy will be enrolled.

Based on results of ESCAPE-NA1 and assuming a 50% overall responder rate for the placebo group, there will be approximately 91.3% power to detect an 11.4% absolute effect difference between response rate (proportion of responders, with Day 90 mRS in the range 0 to 2) with nerinetide and placebo, at alpha level 0.025 one-sided (0.05 2-sided), using the planned sample size of 850 evaluable subjects, randomized 1:1, per group [EaST v6.5, 2022].

This sample size was calculated taking into account a dropout/non-evaluable rate of 2% with a lag to primary endpoint assessment of 4 months, estimated accrual rate of 50 participants per month, target effects 0.5 vs 0.6114 and a single interim analysis for early superiority stopping with an O'Brien-Fleming alpha spending function boundary at 60% information (510 evaluable participants). The interim analysis is planned to take place at 60% information (primary endpoint), i.e. when approximately 510 of the target 850 patients have reached their primary endpoint assessment. The cumulative alpha spent at the interim analysis is 0.004 and final analysis 0.025, one-sided (0.05 2-sided); the stopping boundaries on the Z scale are 2.668 (interim) and 1.981 (final) and on the p-value scale 0.004 (interim) and 0.021 (final), all on the assumption that the interim is conducted at 60% information.

See Section 9.4 for further details on the interim analysis.

9.2 Analysis Sets

For purposes of analysis, the following populations are defined:

Population	Description
Enrolled	All participants randomized into the trial.
Randomly Assigned to Study Intervention	All participants who received a dose or partial dose of study drug

Intent to Treat (ITT)	All participants randomized into the trial with grouping by randomized treatment, regardless of treatment actually received. Participant grouped according to the randomized (intended) treatment.
Per Protocol (PP)	All participants randomized and treated, with no major protocol deviations including: did not meet inclusion/exclusion criteria, did not receive planned dose volume, incorrect study drug vial, infusion more than 15 minutes, consent not obtained.
Safety	All participants randomly assigned to study intervention and who receive any volume of study drug. Participants will be analyzed according to the intervention they actually received.

The primary efficacy estimand analysis will be conducted in the ITT population. The primary efficacy estimand analysis will be repeated on the Per Protocol (PP) population. An ITT analysis will also be conducted for the secondary endpoint estimands, with participant grouped according to the randomized (intended) treatment.

9.3 Statistical Analysis

The statistical analysis plan will be finalized prior to any interim analysis and it will include a more technical and detailed description of the statistical analyses described in this section. In the event that there are any inconsistencies between this protocol and the statistical analysis plan, the statistical analysis will be conducted as per the statistical analysis plan. This section is a summary of the planned statistical analyses of the most important estimand endpoints including primary and key secondary endpoints.

9.3.1 General Considerations

All imputed values will be determined prior to database lock and conducting primary analyses via rules documented prospectively in the final Statistical Analysis Plan for the study.

Deceased participant will score 6 on the mRS, 42 on the NIHSS and 0 on Barthel Index and be counted as non-responders. Every effort will be made to keep missing data, particularly the Day 90 outcome assessments, to a minimum.

For the primary analysis, for participants who are missing the mRS at Day 90:

- if the participant is known to be dead at Day 90, they will be considered to be a non-responder and the mRS will be imputed as 6
- if the mRS was obtained at the Day 30 assessment or later, it will be carried forward as the Day 90 mRS value
- if both the Day 30 and Day 90 mRS scores are missing but the participant is documented to be alive at Day 90 they will be considered to be a non-responder and the mRS will be imputed as a 5
- if both the Day 30 and Day 90 mRS scores are missing and the mortality status of the participant is unknown at Day 90 they will be considered to be a non-responder and the mRS will be imputed as a 6.

If more than 5% subjects randomized are missing the mRS score at Day 90, additional imputation methods will be employed- details of which will be included in the SAP.

For the secondary analysis of rate of mortality, for participants for whom the morality status is not known at Day 90:

- if they were alive at Day 30, then the subject will be imputed as alive at Day 90
- If both the Day 30 and Day 90 mortality is status is missing, the participant will be imputed as Dead at Day 90.

For other analyses involving the mRS (other than mortality), the same imputation approach will be taken as for the primary analysis. No imputation will be done on the missing one-year follow-up mortality data.

A number of events may occur after study drug initiation and during the 90-day trial observation period that could impact data completeness, the interpretation of, or the ability to observe, each outcome variable. Details of handling of such events, which may or may not meet the ICH definition of intercurrent events (IEs)[42], will be provided in the SAP.

Participants who are missing NIHSS or BI endpoint data at Day 90 will have the last recorded score carried forward, provided that this score was obtained at the Day 30 visit or later. Otherwise, the missing NIHSS or BI will be imputed to the median score obtained at Day 90 in the trial.

Sensitivity analyses using various imputation techniques will be specified prospectively in the SAP before the database lock for the interim analysis if more than 5% of participants randomized are missing the primary endpoint.

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 participants 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 one-sided 0.025 (2-sided 0.05).

In order to avoid sparse sites (sites with fewer than 10 randomized participants) sites within a geographic region (Canada/USA/Rest of World) with fewer than 10 randomized participants will be pooled into a single pooled site for use in efficacy analyses.

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

9.3.2 Efficacy Analysis

The primary and secondary efficacy estimand analyses will be conducted on the intent-to-treat (ITT) population, defined as all participants randomized into the trial with grouping by randomized treatment, regardless of treatment actually received. Two additional 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.

In order to protect the overall trial false positive rate, the primary efficacy estimand analysis and secondary efficacy estimand analyses 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 estimand analysis.
2) Secondary efficacy estimand analyses, as specified in the order presented in Table 9-1 below.

Table 9-1: Statistical Analysis Methods

Endpoint	Statistical Analysis Methods
Primary	Overall proportion participants experiencing a favorable functional outcome 90-days post-randomization, defined as 0 to 2 on the mRS. The pivotal primary analysis will be conducted on the ITT population at the one-sided 0.025 (2-sided 0.05) significance level overall (for the trial), adjusted for the interim analysis per the O'Brien-Fleming boundary spending function. It will use the method of Ge et al, 2011[40]. The proportion difference in responder rate between two treatment groups and the associated standard error and 95% confidence interval will be reported. P-value for the primary endpoint will be calculated based on the estimates reported by the Ge et al, 2011[40] method. The pivotal primary analysis model will be a logistic regression model with fixed effects including the treatment group, the stratification covariate of time from stroke onset to randomization ≤ 4.5 hours (yes/no) and the randomized minimization factors (age, baseline NIHSS score, occlusion location, time from qualifying imaging to randomization, baseline ASPECT score, sex, and pooled site), and an interaction term of treatment by time from stroke onset to randomization. If the interaction term is not significant at the level of 0.05, it will be removed from the model. The odds ratios along with the 95% CI will be reported in addition to the primary Ge et al, 2011 method results. If the interaction term is significant, results will be also reported by randomization stratum separately, using the Ge et al, 2011 method. A secondary analysis will be conducted in addition to the adjusted primary analysis to provide additional insights into the treatment effect (i.e. estimation of unadjusted treatment effects).

	The secondary analysis in support of the primary analysis will be the two sample proportion Wald test. The risk difference along with the estimated standard error, 95% CI and the Wald test statistics will be reported. An additional analysis will be conducted in which the primary analysis is reapplied to the per protocol population with observed cases only. Three separate efficacy analysis timepoints are planned for this trial. The first analysis will be at the interim analysis planned at 60% information on the primary endpoint. The second analysis timepoint will be based on the completion of Day 90 visits for the main trial. The third analysis will be following the completion of the 1-Year follow up.
Secondary	The secondary endpoints with binary outcomes (mortality rate, worsening of stroke and NIHSS responder) will be assessed via the same analysis as the primary analysis (i.e, the logistic regression based, with the odds ratios along with the 95% CI and two sample proportion test) All tests will be conducted with one-sided significance level of 0.025 (2-sided 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, secondary analysis second, etc.), after the first test which fails. The mRS shift analysis will only be conducted as part of the fixed sequence testing sequence provided that the proportional odds assumption is found to be valid on testing. If it is found to be invalid, the remaining secondary endpoints will be deemed to analyzed in the fixed testing sequence specified without the mRS shift analysis; i.e. it will be removed from the fixed sequence testing sequence.
	Mortality rates, defined as the number of deaths observed divided by the number of participants observed over the 90-day study period, will be compared between nerinetide and placebo control participants on the ITT set. Results will be summarized and tabulated., Mortality analysis will be additionally supported using time-to-death survival function analysis, both unadjusted shown using Kaplan-Meier analysis and adjusted via Cox proportional hazards regression.
	Worsening of Stroke is determined as the number of participants experiencing at least one worsening of stroke divided by the number of participants observed over the 90-day period in that treatment group, between nerinetide and placebo control participants. Results will be summarized and tabulated.
	"mRS shift analysis" The primary 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 nerinetide 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% CI). 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. An adjusted POM will be used to derive the common odds of improvement (i.e. the nerinetide vs. placebo "shift" in mRS score distributions). Adjustment of the treatment effects will include the same set of variables as used for the logistic regression model for the primary estimand. Deceased participants will be included with a mRS score of 6. Since the 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 secondary analysis will be removed from the fixed sequence testing procedure and conducted as an exploratory analysis instead.
	NIHSS scores at Day 90 will be dichotomized into 0-2 (indicating a good neurological outcome) versus >2 (indicating otherwise). Deceased participants will be included with a NIHSS score of 42. The proportion of participants achieving a good neurological outcome at Day 90 in nerinetide versus placebo control participants will be analyzed. Results will be summarized and tabulated.
Tertiary	**Total volume of new strokes** on MRI or CT brain imaging in the nerinetide versus placebo control participants. Total volume will be assessed using an unadjusted two-tailed Student's t-test and supported by an adjusted analysis comprising of a linear regression that includes the stratification and minimization variables. A cubic root transformation will be performed if needed. Results will be reported with confidence intervals for the unadjusted and adjusted treatment effect between treatment groups.
	Barthel, Day 90 mRS≤1, Day 90 mRS 4-6, the tertiary outcomes comprising proportions of responders will be analyzed similarly to the primary efficacy analysis. Deceased participants will be included in the ITT population with a Barthel Index score of 0. **EQ-5D-5L** will be assessed descriptively. Results will be summarized and tabulated.
Exploratory	Exploratory subgroup analyses will be conducted to determine whether any of these factors can modify the effect of the nerinetide 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: • Time from stroke onset to randomization (≤4.5 hours vs. > 4.5 hours) • Age (<80 years vs ≥ 80 years of age) • Age (<65 years vs ≥ 65 years of age) • Sex (men vs. women)

	• Ethnicity (Hispanics vs non-Hispanic) • Race (White, Asian, Black, Other) • Baseline stroke severity (NIHSS > Median vs. NIHSS <= Median) • Baseline occlusion location (MCA vs. ICA) • Baseline ASPECT score (5-7 vs. 8-10) • Time from qualifying imaging to study drug initiation (greater than vs. less than the median). • Time from onset of stroke symptoms to start of study drug (greater than vs. less than the median). • Degree of reperfusion (TICI 2b-3 vs. TICI <2b) • Subjects weighing between 105-120 kg. vs. ≤ 105 kg. • Subjects receiving a thrombolytic treatment post-randomization vs. those that do not. • Subjects diagnosed with COVID-19 infection while in the acute care hospital or known to have active COVID-19 infection at presentation vs. those that do not. • Subjects who received a thrombolytic as a rescue medication vs. those that do not • Subjects in whom there was a reported AE of recurrent stroke vs. those in who there was not Additional sub-groups may be examined. Sub-group analysis results will also be reported by randomization strata if the interaction term in the primary pivotal analysis plus the treatment-by-stratification interaction term is significant at the 0.05 level in the adjusted logistic regression of the primary analysis.

9.3.2.1 Adjustment for Covariates

Adjustment will be conducted as described in Table 9-1. Full details will be specified in detail in the SAP.

9.3.2.2 Outcome Analysis for 1-Year Sub-Study

The outcomes of the 1-Year Sub-Study are intended to be supportive of the 90-day outcomes of the main study. The analysis population will be comprised of participants with a valid consent for the 1-year sub-study in the relevant jurisdiction. Missing data will be imputed as per Section 9.3.1. A summary table will specify the differences between the population analyzed in the main study and the 1-year sub-study, including deaths, losses to follow-up and withdrawal of consent.

The primary outcome is the proportion of subjects with independent functioning on the modified Rankin Scale (mRS), as defined by a score of 0-2) at 1 year.

The secondary outcomes include:

- A reduction in mortality rate, as defined by event rate (%) for mortality over the 1-year study period.
- The proportion of subjects with independent function on activities of daily living defined on the modified Barthel Index (BI) with a score of ≥ 95 at 1 year.
- Health-related quality of life, as measured by the EQ-5D-5L at 1 year.

9.3.3 Analyses of Safety

All safety analyses will be performed on the Safety Population. The main analyses will be frequency of SAEs and 90-day mortality.

Endpoint	Statistical Analysis Methods
Serious Adverse Events	The frequency of SAEs will be summarized using the Medical Dictionary for Regulatory Activities (MedDRA) Version 23.1.
SAEs leading to death	The frequency of fatal SAEs will be summarized using the Medical Dictionary for Regulatory Activities (MedDRA) Version 23.1.
Adverse Events	The frequency of AEs will be summarized using the Medical Dictionary for Regulatory Activities (MedDRA) Version 23.1.
Vital Signs	Absolute values and changes for vital signs from pre-dose to Day 2 will be documented. The maximum deviation of BP from Baseline between drug and placebo control groups (systolic and diastolic) to Day 2 will be analyzed.
Lab Safety	Absolute values for laboratory results will be summarized descriptively.
Prior and Concomitant Medications	Prior and concomitant medications will be summarized using the WHO Drug Dictionary.

9.3.4 Pharmacokinetics

Descriptive statistics will be calculated for plasma concentrations and for all PK parameters (nerinetide). In addition, dose proportionality and linearity of dose dependent PK parameters will be investigated. Individual and mean plasma concentration versus time curves will be plotted on linear and semi-logarithmic scales. Plasma concentration versus time curves will be labelled appropriately with treatment regimen and batch number.

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

- **AUC_{0-t}:** Area under the concentration-time curve from time zero to time of last measurable concentration
- **AUC_{0-inf}:** Area under the concentration-time curve from time zero to infinity
- **C_{max}:** Maximum plasma concentration observed after dosing
- **T_{max}:** Time to occurrence of C_{max}

- **t½:** Terminal elimination half-life

Samples with no detectable nerinetide will be excluded from analysis (placebo).

9.4 Interim Analysis

There will be an interim efficacy analysis in this trial. It will be conducted by the unblinded statistician in the Independent Statistical Group.

The interim efficacy analysis will be performed after approximately 510 participants have complete the Day 90 follow-up, at 60% information on the primary endpoint. The planned sample size is 850 participants randomized 1:1, allowing for a single interim analysis at 60% information (when about 510 patients have primary endpoint assessments) with O'Brien-Fleming alpha-spending function stopping boundary for overwhelming efficacy. The cumulative alpha spent at the interim analysis is 0.004 and final analysis 0.025, 1-sided; the stopping boundaries on the Z scale are 2.668 (interim) and 1.981 (final) and on the p-value scale 0.004 (interim) and 0.021 (final), all on the assumption that the interim is conducted at 60% information .

These calculations were performed by taking into account a dropout/non-evaluable rate of 2% with 4 month lag to primary endpoint assessment, estimated accrual rate of 50 participants per month, target effects 0.5 vs 0.6114 and a single interim analysis for early superiority stopping with an O'Brien-Fleming alpha spending function boundary at 60% information (510 evaluable participants). [EaST v6.5, 2022].

The IDMC may recommend stopping for overwhelming efficacy at the interim analysis if the test statistic crosses the O-F boundary. The IDMC Charter will provide further details on the rationale for, and how, these recommendations will be communicated.

10 SUPPORTING DOCUMENTATION and OPERATIONAL CONSIDERATIONS

10.1 Appendix 1: Regulatory, Ethical, and Study Oversight Considerations

10.1.1 Regulatory and Ethical Considerations

This study will be conducted in accordance with the protocol and with the following:

- Consensus ethical principles derived from international guidelines including the Declaration of Helsinki and Council for International Organizations of Medical Sciences (CIOMS) International Ethical Guidelines
- Applicable ICH Good Clinical Practice (GCP) Guidelines
- Applicable laws and regulations including: Canadian Food and Drug Regulations, and the Canadian Tri-Council Policy Statement on Ethical Conduct for Research involving Humans (2), United States Code of Federal Regulations (CFR; including Title 21 Parts 50, 54, 56, and 312), where applicable.
- Applicable guidelines issued due to the COVID-19 pandemic

The protocol, protocol amendments, ICF, Investigator Brochure, and other relevant documents (e.g., advertisements) must be submitted to an IRB/IEC by the investigator and reviewed and approved by the IRB/IEC before the study is initiated.

Any amendments to the protocol will require IRB/IEC approval before implementation of changes made to the study design, except for changes necessary to eliminate an immediate hazard to study participants.

The investigator will be responsible for the following:

- Providing written summaries of the status of the study to the IRB/IEC annually or more frequently in accordance with the requirements, policies, and procedures established by the IRB/IEC
- Notifying the IRB/IEC of SAEs or other significant safety findings as required by IRB/IEC procedures
- Providing oversight of the conduct of the study at the site and adherence to requirements of 21 CFR, ICH guidelines, the IRB/IEC, European regulation 536/2014 for clinical studies (if applicable), and all other applicable local regulations

10.1.2 Financial Disclosure

As requested to allow the sponsor to submit complete and accurate financial certification or disclosure statements to the appropriate regulatory authorities, investigators and sub-investigators may provide the sponsor with sufficient, accurate financial information. This includes information on financial interests during the course of the study and potentially for one year after completion of the study.

Routine care is expected to be paid for by the existing standard medical insurance system. This will include but is not limited to:

- Admission to hospital
- Baseline laboratory testing, pregnancy test, baseline NCCT and CTA, baseline CTP
- Endovascular procedure and angiography
- Follow-up limited-sequence MR brain imaging at Day 2/3
- Follow-up laboratory testing (other than the mandated tests at Day 2/3)
- Physician fees
- Treatment processes in the endovascular lab since they are considered standard of care
- Stroke unit care in hospital
- Nursing care
- Rehabilitation and home care if relevant
- Outpatient clinic follow-up at 90 days (routine)

The study fees are designed to cover the costs of study personnel, data collection, research study processes and treatments, the 30-day follow up visit, the 90-day follow-up visit, 1-Year follow up contact, CRF completion, adverse event reporting, concomitant medication reporting, submission of imaging to the core lab and support of remote monitoring, if applicable. The study fees are inclusive of any local institutional overhead/indirect costs.

10.1.3 Informed Consent Process

See Section 5.5.2 for further details.

10.1.4 Data Protection

Participants will be assigned a unique identifier by the sponsor. Any participant records or datasets that are transferred to the sponsor will contain the identifier only; participant names or any information which would make the participant identifiable will not be transferred.

The participant must be informed that his/her personal study-related data will be used by the sponsor in accordance with local data protection law. The level of disclosure must also be explained to the participant who will be required to give consent for their data to be used as described in the informed consent

Personal medical information may be reviewed for the purpose of verifying data recorded in the eCRF by the site monitors. Other properly authorized persons, such as the regulatory authorities, may also have access to these records. Personal medical information is always treated as confidential. The participant must be informed that his/her medical records may be examined by Clinical Quality Assurance auditors or other authorized personnel appointed by the sponsor, by appropriate IRB/IEC members, and by inspectors from regulatory authorities.

All imaging, evaluation forms, reports, and other records that leave the site are identified only by the site and participant number to maintain participant confidentiality. All records are kept in a locked file cabinet. Clinical information is not released without written permission of the

participant, except as necessary for monitoring by REB/IRB, Health Canada, the sponsor, or the sponsor's designee.

All study investigators at the clinical sites, monitors and sponsor staff must ensure that the confidentiality of personal identity and all personal medical information of study participants are maintained at all times. Federal legislation in Canada (PIPEDA), U.S (HIPAA), Europe (GDPR) and local legislations must be followed.

10.1.5 Committees Structure

Safety reviews may be performed several times by the Sponsor's staff in the course of the trial. Any questions pertaining to the reported clinical data will be submitted to the investigator for resolution. Each step of this process will be monitored through the implementation of individual passwords to maintain appropriate database access and to ensure database integrity.

10.1.5.1 Independent Data Monitoring Committee

The Independent Statistical Group will perform the efficacy interim analysis after approximately 510 participants complete the Day 90 follow-up. The interim analysis for efficacy will also be assessed by the IDMC which will review unblinded trial safety and efficacy data and make recommendations to the blinded Sponsor regarding continuation or stopping. The IDMC may recommend the trial be stopped for overwhelming efficacy at the interim analysis if the test statistic crosses the O'Brien-Fleming[43].

Activities, mandate, responsibilities, communication structure and function of the IDMC and the Independent Statistical Group will be documented in the IDMC Charter prospectively. A Blinding Plan will also be included, specifying sequestering and blinding measures planned for the trial (including analysis firewalls) to prevent operational bias from revelation outside the IDMC of any aggregate interim results on safety or efficacy by treatment arm.

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 participant blind to results by treatment assignment during the study. Firewalls will be in place at the Statistical Analysis Center preparing all interim reports to protect and sequester all interim results on safety and efficacy. This will be detailed in the IDMC Charter.

10.1.6 Dissemination of Clinical Study Data

Study information and tabular study results will be posted on the US National Institutes of Health's website www.clinicaltrials.gov within one year of study completion.

10.1.7 Data Quality Assurance

All participant data relating to the study will be recorded on the electronic CRF unless transmitted to the sponsor or designee electronically (e.g., imaging). The investigator is responsible for verifying that data entries are accurate and correct signing the CRF. The investigator must maintain accurate documentation (source data) that supports the information entered in the CRF.

The investigator must permit study-related monitoring, audits, IRB/IEC review, and regulatory agency inspections and provide direct access to source data documents. The investigator agrees to

allow the monitor(s) direct access to all relevant documents, and to allocate his/her time and the time of staff to discuss findings, corrective actions and any relevant issues. In addition to contacts during the study, the monitor may also contact the site prior to the start of the study to discuss the protocol and data collection procedures with site personnel.

Except for an emergency situation in which proper care for the protection, safety and well- being of the study participants requires medical treatment, the study will be conducted as described in the approved protocol, ICH-GCP, SOPs and regulatory requirements. All medical treatments will be recorded. Any deviation(s) from the protocol will be recorded and presented in the final clinical study report.

To ensure monitoring responsibilities are performed to the fullest extent possible, industry experienced study monitors will perform on site data verification for the trial. All data monitored on site are verified for accuracy and completeness using source documents for all participants.

The sponsor will determine the extent, nature, and frequency of on-site visits that are needed to ensure that the study is being conducted in accordance with the approved protocol (and any amendments), GCP, and all applicable regulatory requirements Monitoring details describing strategy (e.g., risk-based initiatives in operations and quality such as Risk Management and Mitigation Strategies and Analytical Risk-Based Monitoring), methods, responsibilities and requirements, including handling of noncompliance issues and monitoring techniques (central, remote, or on-site monitoring) are provided in the Monitoring Plan.

The sponsor or designee is responsible for the data management of this study including quality checking of the data. The sponsor assumes accountability for actions delegated to other individuals (e.g., Contract Research Organizations).

Study monitors will perform ongoing source data verification to confirm that data entered into the CRF by authorized site personnel are accurate, complete, and verifiable from source documents; that the safety and rights of participants are being protected; and that the study is being conducted in accordance with the currently approved protocol and any other study agreements, ICH GCP, and all applicable regulatory requirements. This review may occur at the study site or remotely. Any records supplied by the site for the purpose of remote monitoring and remote source document verification must comply with local legislation and the process approved by the local IRB/EC/REB, when required.

Records and documents, including signed ICFs, pertaining to the conduct of this study must be retained by the investigator for 15 years after study completion unless local regulations or institutional policies require a longer retention period. No records may be destroyed during the retention period without the written approval of the sponsor. No records may be transferred to another location or party without written notification to the sponsor.

10.1.8 Source Documents

Source documents provide evidence for the existence of the participant and substantiate the integrity of the data collected. Source documents are filed at the investigator's site. Source documents specification per site will be agreed prior to first participant enrolled at the site.

Data reported in the eCRFs must be consistent with the source documents or the discrepancies must be explained. The investigator may need to request previous medical records or transfer

records, depending on the study. Also, current medical records must be available. Any records supplied by the site for the purpose of remote monitoring and remote source document verification must be de-identified using only the unique trial specific participant identifier. This process will be aligned with local ethics and privacy requirements.

If clinical outcome assessments are collected using the electronic Clinical Outcome Assessment (eCOA) tool data will transferred directly to the eCRF, in this case the eCOA record will be the sole source document for these assessments.

Any investigators shall supply the sponsor, upon request, with any required background data from the study documentation or clinic records. This is particularly important when errors in data transcription are suspected. In case of special problems and/or governmental queries or requests for audit inspections, it is also necessary to have access to the complete study records, provided that participant confidentiality is protected.

Definition of what constitutes source data may include: participant hospital/clinic records, physician's and nurse's notes, appointment book, original laboratory reports, ECG, X-ray, pathology and special assessment reports, signed consent forms, consultant letters, and source worksheets.

The Investigator must maintain adequate and accurate records to enable the conduct of the study to be fully documented and the study data to be subsequently verified. These documents should be classified into two different separate categories: (1) Investigator's Study File; and (2) Participant Clinical Source Documents.

The Investigator's Study File will contain the Protocol/Amendments, CRFs, REB/IRB and governmental approval with correspondence, all versions of ethics approved informed consent forms, staff curriculum vitae and authorization forms and other appropriate documents/correspondence, etc.

The investigator must keep these two categories of documents on file according to local clinical trial regulation. In Canada, all study documents for a regulated trial require storage for 15 years. After that period of time the documents may be destroyed, participant to local regulations.

The Investigator and the sponsor will maintain the records of disposition of the drug and the clinic records in accordance with ICH-GCP and each applicable regulatory agency. Clinic records will be retained at the site until informed by the sponsor to destroy the documents. If the clinical study must be terminated for any reason, the investigator will return all study materials to the sponsor and provide a written statement as to why the termination has taken place and notify the REB/IRB

10.1.9 Study and Site Start and Closure

The study start date is the date on which the clinical study will be open for recruitment of participants. The first act of recruitment is the first site open and will be the study start date.

The sponsor designee reserves the right to close the study site or terminate the study at any time for any reason at the sole discretion of the sponsor, which may include new or emerging safety information that negatively affects the benefit/risk profile of the drug.

Study sites will be closed upon study completion at 1-Year. A study site is considered closed when all required documents and study supplies have been collected and a study-site closure visit has been performed.

The investigator may initiate study-site closure at any time, provided there is reasonable cause and sufficient notice is given in advance of the intended termination.

Reasons for the early closure of a study site by the sponsor or investigator may include but are not limited to:

- Failure of the investigator to comply with the protocol, the requirements of the REB/IRB or local health authorities, the sponsor's procedures, or GCP guidelines
- Inadequate recruitment of participants by the investigator
- Discontinuation of further study intervention development

If the study is prematurely terminated or suspended, the sponsor shall promptly inform the Investigators, the REBs/IRBs, the regulatory authorities, and any contract research organization(s) used in the study of the reason for termination or suspension, as specified by the applicable regulatory requirements. The Investigator shall promptly inform the participant and should assure appropriate participant therapy and/or follow-up

10.1.10 Publication Policy

The results of this study may be published or presented at scientific meetings. If this is foreseen, the investigator agrees to submit all manuscripts or abstracts to the sponsor before submission. This allows the sponsor to protect proprietary information and to provide comments.

The sponsor will permit any and all academic publications arising from the trial data provided that no publication containing unblinded trial data precedes publication of the overall trial results in a peer-review journal, and are (1) approved by the trial executive committee and (2) the publication authors notify the sponsor at least 30 days prior to submittal for publication with a copy of such proposed publication for the sponsor’s review and comment. Employees or consultants of the sponsor will only be named as authors in any such publication if the parties agree that it is appropriate under the usual conventions used by academic institutions for naming authors in scientific publications. Upon request of the sponsor the publication or disclosure shall be delayed for up to 60 days in order to allow for the filing of a patent application.

The sponsor will comply with the requirements for publication of study results. In accordance with standard editorial and ethical practice, the sponsor will generally support publication of multicentre studies only in their entirety and not as individual site data. In this case, a coordinating investigator will be designated by mutual agreement.

A trial executive committee shall be formed and include at least the trial principal investigator and co-principal investigator, the statistical consultant, and representatives of the Sponsor. The trial executive committee will be co-authors on all publications and presentations. The primary author list for the primary publication will consist of the executive committee and the site principal/qualified investigator at each of the sites. A formal publication policy will be presented and developed by the trial executive.

Authorship will be determined by mutual agreement and in line with International Committee of Medical Journal Editors authorship requirements.

10.1.11 Audits and Inspections

In accordance with the principles of ICH E6 Guideline for Good Clinical Practice, the study site may be inspected by regulatory authorities and/or audited by NoNO Inc. Quality Assurance (QA) or their designates. The investigator and relevant clinical support staff will be required to be actively involved in audits and inspections, including staff interviews, and to make all necessary documentation and data available upon request.

During the course of the study and/or after it has been completed, one or more investigator site audits may be undertaken by auditors from NoNO QA or delegates. The purpose of these audits is to determine whether or not the study is being/has been conducted and monitored in compliance with recognized ICH E6 Guideline for Good Clinical Practice, protocol and approved amendment requirements, applicable local SOPs, and local laws and regulations. It is the responsibility of the investigator and site staff to promptly address, by coordinating with NoNO Inc. any deficiencies stemming out of regulatory inspections and NoNO QA or delegate audits, and to ensure that agreed-upon corrective and preventive actions are implemented as soon as possible.

An inspection by any regulatory authority may occur at any time during or after completion of the study. If an investigator is contacted by a regulatory authority for the purpose of conducting an inspection or to discuss any compliance issues, he/she is required to contact NoNO Inc immediately.

10.2 Appendix 2: Clinical Laboratory Tests and Imaging

10.2.1 Clinical Laboratory Tests

The tests detailed in Table 10-1 will be performed as per local hospital laboratory.

Table 10-1: Protocol-Required Safety Laboratory Assessments

Laboratory Tests	Parameters
Hematology	Platelet Count Hemoglobin Hematocrit
Chemistry	Serum creatinine Serum glucose
Electrolytes	Sodium Potassium Chloride
Pregnancy testing	Highly sensitive (Serum or urine) human chorionic gonadotropin (hCG) pregnancy test (as needed for women of childbearing potential)

10.2.2 Imaging

At baseline all participants will undergo brain imaging for assessment of inclusion into the trial as described in Section 5.5.1. The Day 2/3 MR will be used to assess infarct volume (including a minimum of axial DWI, gradient-echo (GRE), FLAIR). The Day 2/3 MR is considered a standard of care imaging procedure. If MR is unavailable, then NCCT is allowed.

The baseline and all brain/neurovascular imaging conducted up to the Day 2/3 MR will be rendered anonymous and sent to the central core imaging lab. The core imaging lab staff will review the imaging against the imaging inclusion/exclusion criteria found in Sections 5.1 and 5.2in order to ensure adherence to the imaging guidelines and enrollment criteria for training purposes. Centrally adjudicated imaging will be used to determine baseline ASPECT scores, adherence to qualifying imaging criteria, as well as, reperfusion rates (TICI scores) and volume of stroke at Day 2/3. Other brain imaging will be sent only if requested by the adjudication committee.

For all interval times assessed from imaging, the time zero will be the first slice of the NCCT scan. Imaging date and time will be collected in the CRF.

Notes:

Sites that did not participate in the ESCAPE-NA1 trial will submit sample images to the imaging core lab for quality assessment.

Baseline imaging may be completed at a hospital affiliated with the trial site.

10.3 Appendix 3: Adverse Events: Definitions and Procedures for Recording, Evaluating, Follow-up, and Reporting

10.3.1 Definition of AE

AE Definition
An AE is any untoward medical occurrence in a patient 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.
Adverse Drug Reaction (ADR) In the pre-approval clinical experience with a new medicinal product or its new usages, particularly as the therapeutic dose(s) may not be established: all noxious and unintended responses to a medicinal product related to any dose should be considered adverse drug reactions. The phrase "responses to a medicinal product" means that a causal relationship between a medicinal product and an adverse event is at least a reasonable possibility, i.e., the relationship cannot be ruled out. ***Unexpected Adverse Drug Reaction*** An adverse reaction, the nature or severity of which is not consistent with the applicable product information (e.g., Investigator's Brochure for an unapproved investigational medicinal product).
Events <u>Meeting</u> the AE Definition
• A new illness • The worsening of a concomitant illness • Any abnormal laboratory test results (hematology, clinical chemistry, or urinalysis) or other safety assessments (e.g., ECG, radiological scans, vital signs measurements), including those that worsen from baseline, considered clinically significant in the medical and scientific judgment of the investigator (i.e., not related to progression of underlying disease). • Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition. • New conditions detected or diagnosed after study intervention administration even though it may have been present before the start of the study. • Signs, symptoms, or the clinical sequelae of a suspected drug-drug interaction. • Signs, symptoms, or the clinical sequelae of a suspected overdose of either study intervention or a concomitant medication. Overdose per se will not be reported as an AE/SAE unless it is an intentional overdose taken with possible suicidal/self-harming intent. Such overdoses should be reported regardless of sequelae.

10.3.2 Definition of SAE

A 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. Is 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.

10.3.3 Recording and Follow-Up of AE and/or SAE

AE and SAE Recording

- When an AE/SAE occurs, it is the responsibility of the investigator to review all documentation (e.g., hospital progress notes, laboratory reports, and diagnostics reports) related to the event.
- The investigator will then record all relevant AE/SAE information in the CRF.
- It is **not** acceptable for the investigator to send photocopies of the participant's medical records to the Sponsor in lieu of completion of the CRF pages and SAE reports.
- There may be instances when copies of medical records for certain cases are requested by the Sponsor. In this case, all participant identifiers, with the exception of the participant study identification number, will be redacted on the copies of the medical records before submission to the Sponsor.
- The investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. Whenever possible, the diagnosis (not the individual signs/symptoms) will be documented as the AE/SAE.

Assessment of Intensity

The investigator will make an assessment of intensity for each AE and SAE reported during the study and assign it to 1 of the following categories:

- Mild: Awareness of sign or symptom but easily tolerated.
- Moderate: Discomfort sufficient to cause interference with normal activities.
- Severe: Incapacitating, with inability to perform normal activities.

An AE that is assessed as severe should not be confused with a SAE. Severe is a category utilized for rating the intensity of an event; and both AEs and SAEs can be assessed as severe. An event is defined as 'serious' when it meets at least 1 of the predefined outcomes as described in the definition of an SAE, NOT when it is rated as severe.

Assessment of Causality

- The investigator is obligated to assess the relationship between study intervention and each occurrence of each AE/SAE.
- A "reasonable possibility" of a relationship conveys that there are facts, evidence, and/or arguments to suggest a causal relationship, rather than a relationship cannot be ruled out.

- The investigator will use clinical judgment to determine the relationship.
- Alternative causes, such as underlying disease(s), concomitant therapy, and other risk factors, as well as the temporal relationship of the event to study intervention administration will be considered and investigated.
- The investigator will also consult the Investigator's Brochure (IB) and/or Product Information, for marketed products, in his/her assessment.
- For each AE/SAE, the investigator **must** document in the medical notes that he/she has reviewed the AE/SAE and has provided an assessment of causality.
- There may be situations in which an SAE has occurred and the investigator has minimal information to include in the initial report to the Sponsor. However, it is very important that the investigator always make an assessment of causality for every event before the initial transmission of the SAE data to the Sponsor.
- The investigator may change his/her opinion of causality in light of follow-up information and send a SAE follow-up report with the updated causality assessment.
- The causality assessment is one of the criteria used when determining regulatory reporting requirements.

AE Causality/ Relationship	
Related	A clinical event, including laboratory test abnormality, where there is a "reasonable possibility" that the SAE was caused by the study drug, meaning that there is evidence or arguments to suggest a causal relationship.
Possibly:	A clinical event, including laboratory test abnormality, with a reasonable time sequence to drug administration, but which could also be explained by concurrent disease or other drugs or chemicals. Information on drug withdrawal may be lacking or unclear.
Unrelated:	This category is applicable to AEs which are judged to be clearly and incontrovertibly due to extraneous causes (diseases, environment, etc.) and do not meet the criteria for drug relationship listed for the above-mentioned conditions.

Follow-up of AEs and SAEs

- The investigator is obligated to perform or arrange for the conduct of supplemental measurements and/or evaluations as medically indicated or as requested by the Sponsor to elucidate the nature and/or causality of the AE or SAE as fully as possible. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals.
- If a participant dies during participation in the study the investigator may be requested by the Sponsor to provide the Sponsor with a copy of any post-mortem findings including histopathology.
- New or updated information will be recorded in the originally completed CRF.

- The investigator will submit any updated SAE data to the Sponsor within 24 hours of receipt of the information.

10.3.4 Reporting of SAEs

SAE Reporting to the Sponsor via an Electronic Data Collection Tool
• The primary mechanism for reporting an SAE to the Sponsor will be the electronic data collection tool. • If the electronic system is unavailable, then the site will use the paper SAE data collection tool (see next section) **in order to report the event within 24 hours**. • The site will enter the SAE data into the electronic system as soon as it becomes available. • After the study is completed at a given site, the electronic data collection tool will be taken off-line to prevent the entry of new data or changes to existing data. • If a site receives a report of a new SAE from a study participant or receives updated data on a previously reported SAE after the electronic data collection tool has been taken off-line, then the site can report this information on a paper SAE form or to the Drug Safety Department at NoNO by telephone or email. 1-416-583-1687 or sae@nonoinc.ca
SAE Reporting to the Sponsor via Paper CRF
If the eCRF system is not available, a paper SAE form should be directed within 24 hours to: Drug Safety at NoNO Inc. sae@nonoinc.ca or 1-416-583-1856 (for fax) Initial notification via telephone does not replace the need for the investigator to complete and sign the SAE CRF pages within the designated reporting time frames.

10.4 Appendix 4: Collection of Pregnancy Information

Male participants with partners who become pregnant

The investigator will attempt to collect pregnancy information on any male participant's female partner who becomes pregnant while the male participant is in this study. This applies only to male participants who receive study drug.

After obtaining the necessary signed informed consent from the pregnant female partner directly, the investigator will record pregnancy information on the Pregnancy Reporting Form and submit it to the sponsor within 24 hours of learning of the partner's pregnancy. The female partner will also be followed to determine the outcome of the pregnancy. Information on the status of the mother and child will be forwarded to the sponsor. Generally, the follow-up will be no longer than 4 weeks following the estimated delivery date. Any termination of the pregnancy will be reported regardless of fetal status (presence or absence of anomalies) or indication for the procedure.

Female Participants who become pregnant

The investigator will collect pregnancy information on any female participant who becomes pregnant while participating in this study. The initial information will be recorded on the Pregnancy Reporting Form and submitted to the sponsor within 24 hours of learning of a participant's pregnancy.

The participant will be followed to determine the outcome of the pregnancy. The investigator will collect follow-up information on the participant and the neonate and the information will be forwarded to the sponsor. Generally, follow-up will not be required for longer than 4 weeks beyond the estimated delivery date. Any termination of pregnancy will be reported, regardless of fetal status (presence or absence of anomalies) or indication for the procedure.

While pregnancy itself is not considered to be an AE or SAE, any pregnancy complication or elective termination of a pregnancy for medical reasons will be reported as an AE or SAE.

A spontaneous abortion (occurring at <22 weeks gestational age) or still birth (occurring at >22 weeks gestational age) is always considered to be an SAE and will be reported as such.

Any post-study pregnancy related SAE considered reasonably related to the study intervention by the investigator will be reported to the sponsor as described in Section 8.3.4. While the investigator is not obligated to actively seek this information in former study participants, he or she may learn of an SAE through spontaneous reporting.

Any female participant who becomes pregnant while participating in the study will discontinue study intervention but will continue to be followed to the end of the trial.

10.5 Appendix 5: Abbreviations

AHA	American Heart Association
AIS	Acute Ischemic Stroke
AE	Adverse Event
AESI	Adverse Event of Special Interest
ASPECTS	Alberta Stroke Program Early Computerized Tomography Score
β-hCG	Beta-human Chorionic Gonadotropin
BI	Barthel Index
BP	Blood Pressure
CBC	Complete Blood Count
CFR	Code of Federal Regulations
CI	Confidence Interval
CIOMS	Council for International Organizations of Medical Sciences
CRA	Clinical Research Associate
CRF	Case Report Form
CT	Computed Tomography
CTA	Computed Tomographic Angiography
CTP	Computed Tomographic Perfusion
DWI	Diffusion Weighted Imaging
EC	Ethics Committee
eCOA	Electronic Clinical Outcome Assessment
eCRF	Electronic Case Report Form
eMCAO	Embolic Middle Cerebral Artery Occlusion
ESCAPE-NEXT	**E**xtension of **S**troke **C**are with **A**djuvant neuro**P**rotection to **E**ndovascular treatment with **N**erinetide **EX**cluding **T**hrombolysis
EQ-5D-5L	EuroQol health-related quality of life
EVT	Endovascular Thrombectomy
FDA	Food and Drug Administration
FLAIR	Fluid Attenuated Inversion Recovery
GCP	Good Clinical Practice
GDPR	General Data Protection Regulation
HIPAA	Health Insurance Portability and Accountability Act
HR	Heart Rate
ICA	Internal Carotid Artery
ICF	Informed Consent Form
ICH-GCP	International Conference on Harmonization-Good Clinical Practice
IDMC	Independent Data Monitoring Committee
IRB	Institutional Review Board
ITT	Intent-to-treat
IV	Intravenous
IWRS	Interactive Web Response System
LAR	Legally Authorized Representative
LVO	Large Vessel Occlusion
MAP	Mean Arterial Pressure

MCA	Middle Cerebral Artery
MCAO	Middle Cerebral Artery Occlusion
mCTA	Multiphase Computed Tomography Angiography
MedDRA	Medical Dictionary for Regulatory Activities
MR	Magnetic Resonance
MRI	Magnetic Resonance Imaging
mRS	Modified Rankin Scale
n	Number of Observations
NaCl	Sodium Chloride
NCCT	Non-contrast Computed Tomography Scan
NIHSS	National Institutes of Health Stroke Scale
NMDA	N-methyl D-aspartate
NMDAR	N-methyl D-aspartate Receptor
nNOS	Neuronal Nitric Oxide Synthase
NO	Nitric Oxide
O-F	O'Brien Fleming
pH	Potential Hydrogen
PI	Principal Investigator
PIPEDA	Personal Information and Portable Electronic Documents Act
PK	Pharmacokinetic
POM	Proportional Odds Method
PP	Per Protocol
PSD-95	Post-synaptic Density 95 Protein
QA	Quality Assurance
REB	Research Ethics Board
RR	Risk Ratio
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SD	Standard Deviation
SOC	System Organ Class
SOP	Standard Operating Procedures
SUSAR	Suspected Unexpected Serious Adverse Reaction
TICI	Thrombolysis in Cerebral Infarction Score
tMCAO	Temporary Middle Cerebral Artery Occlusion
USA/US	United States of America
VAS	Visual Analogue Scale
WHO	World Health Organization

10.6 Appendix 6: Country-specific Requirements

10.6.1 Addition to Consent Process- Section 5.5.2

The first option for obtaining consent will be signed informed consent from the participant or LAR prior to any protocol-specific procedures. In accordance with ICH GCP, in a life-threatening situation, in the event where informed consent is not feasible, the decision to enroll a subject may be made by the investigator after consultation with an independent physician who is not otherwise participating in the trial provided that it is allowed by applicable national laws and regulations and an approved/favourable opinion by the applicable Research Ethics Board/Ethics Committee. Any decision made will have considered the patient's presumed will.

All methods of conducting the informed consent process must comply with ICH GCP E6. Approval for all method(s) of obtaining informed consent as well as the forms used must be obtained prior to implementation.

In the event the subject remains incapacitated, consent to continue participation in the trial should be obtained from the LAR as soon as it is possible and reasonable.

10.6.2 Addition to Remote Source Document Verification - Section 10.1.7

Remote source data verification (rSDV) will only occur at study sites where the country regulations allow and prior approval has been granted by the regulatory authority, as applicable. In Germany, rSDV will not occur at study sites.

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12 INVESTIGATOR'S AGREEMENT

I have read the attached protocol: **A Multicentre, Randomized, Double-blinded, Placebo-controlled, Parallel Group, Single-dose Design to Determine the Efficacy and Safety of Nerinetide in Participants with Acute Ischemic Stroke Undergoing Endovascular Thrombectomy Excluding Thrombolysis** (ESCAPE-NEXT Trial) (Version number in the header above) and agree to abide by all provisions set forth therein.

I agree to comply with the current International Conference on Harmonisation Guidelines for Good Clinical Practice and the laws, rules, regulations and guidelines of the community, country, state or locality relating to the conduct of the clinical study.

I also agree that persons debarred from conducting or working on clinical studies by any court or regulatory agency will not be allowed to conduct or work on studies for the sponsor.

Name Site Principal Investigator	Signature
Name of Clinical Site	Date

13 ADDENDUM

13.1 COVID-19 Considerations

Improving Access to Stroke Care due to Changes in COVID-19

Based on the clinical data available for nerinetide to date, treatment with nerinetide may slow the progression of ischemic brain damage, providing more time during which endovascular thrombectomy may be of benefit to the patient. This is of even greater relevance during the global COVID-19 pandemic for the following reasons:

(a) There is a necessity to protect hospital staff, resulting in additional hospital protocols involving sanitations and personal protective equipment that minimize the exposure of the clinical stroke team to a potentially COVID-19 positive stroke patient (there is no time for COVID-19 testing to be completed).

(b) There is a necessity to protect the patient, resulting in additional hospital protocols involving distancing him/her from hospital staff and sanitizing equipment (e.g., CT scanner) used on other patients.

These necessities slow down emergency stroke care workflows, potentially causing undue delays in the emergency stroke care of all AIS patients, not just those who may have been exposed to COVID-19. Thus, the possibility that nerinetide, by slowing the progression of ischemic brain damage, mitigates such delays may be of direct benefit to treated patients.

Minimizing Risk to Participants and Staff

The protocol includes the following adaptations to minimize risk while prioritizing the overall well-being and best interests of all involved in the trial. This protocol was written considering the impact of COVID-19 on trial participants, site staff and sponsor staff. With these priorities in mind, the protocol design will still permit assessment of safety and efficacy of nerinetide.

- **Minimize the number of study visits** to align with the trial endpoints
- **Minimize the number of trial specific activities**. The trial was designed to align with standard of care protocols for acute stroke. For example, obtaining data from standard of care assessments conducted during routine stroke care, for the collection and reporting of some safety outcomes (vital signs, laboratory results) and imaging outcomes.
- **Conduct visits by telemedicine or by telephone** at Day 30 and Day 90, where permitted, when a participant is unable to attend the site physically. This will permit the timely collection of efficacy endpoints (mRS, NIHSS, Barthel Index and EQ-5D-5L) and safety data (AE and SAE collection). If the contact is made by phone, only the NIHSS assessment will not be completed, all other assessments will be obtained.
- **Use of remote electronic consent,** where permitted, to obtain initial consent from the LAR, who may not be able to enter the hospital, and to obtain regained capacity consent from participants who did not consent prior to their discharge from hospital.
- **Use of electronic Clinical Outcome Assessments (COA)** is optional for the conduct of the mRS, NIHSS, Barthel Index and EQ-5D-5L assessments at Day 30, Day 90 and 1-Year timepoints. If an electronic COA is used, this will permit site staff to collect trial

endpoints even if they are not permitted into the hospital, reduce data entry time and reduce the time on-site for CRAs in order to monitor the data.

In addition to the above items included in the protocol, ongoing risk assessments and monitoring of the COVID-19 situation will be conducted by the sponsor with input from the local investigators at both a site and country level. These ongoing assessments include changes to any of the following:

- Potential impact on trial participants
- Potential impact on trial site staff, including local or central REB/IRBs/ECs
- Potential impact on sponsor/CRO staff conducting site monitoring and central review of data.

The outcome of these ongoing assessments could result in site-specific or country-specific mitigation plans, which could include:

- Suspension of enrollment in that site or country
- Suspension of on-site visits by participants in that site or country, replacing the physical data capture with some remote measures (telephone or telemedicine, where permitted)
- Suspension of on-site visits being conducted by the CRA, replacing the monitoring with remote review of data and telephone contacts with the site
- Other mitigation plans, as appropriate.

13.2 Assessment Questionnaires

Below is a sample of the assessment questionnaires and instructions to be used in the trial.

Modified Rankin Scale (mRS)	Clinician Reported Outcome English example
National Institute of Health Stroke Score (NIHSS)	Clinician Reported Outcome English example.
Barthel Index	Clinician Reported Outcome English example.
EQ-5D-5L	Patient Reported Outcome USA, English Interviewer Administered example See https://euroqol.org/ for device, country, and language specific versions

Statistical Analysis Plan

A Multicentre, Randomized, Double-blinded, Placebo-controlled, Parallel Group, Single-dose Design to Determine the Efficacy and Safety of Nerinetide in Subjects with Acute Ischemic Stroke Undergoing Endovascular Thrombectomy Excluding Thrombolysis

(ESCAPE-NEXT Trial)

Protocol NA-1-009

Product:	Nerinetide
IND:	118,087
Sponsor:	NoNO Inc. 479A Wellington Street West Toronto, Ontario, Canada M5V 1E7
Date	18 November 2021
Version	Final 2.0

SIGNATURES OF APPROVAL

Personal Protected Data

Version History

This Statistical Analysis Plan (SAP) for the trial ESCAPE-NEXT is based on Protocol V5.0, dated 18 November 2021.

Version	Date	Change	Rationale
1.0	03 May 2021	Not Applicable	Original version
2.0	18 Nov 2021	- Updated hierarchical testing order of secondary endpoints: moved the NIHSS responder endpoint to the last.	- The change in testing order of NIHSS endpoint was due to the concern of increased missingness of Day 90 NIHSS scores that may impact statistical power. - NIHSS cannot be conducted over the phone. Due to COVID-19 there is an increase in the number of Day 90 visits being conducted by phone.
		- Updated the primary analysis model for all binary efficacy endpoints: a generalized linear regression model with adjustment for baseline covariates and randomization stratification factor will be used - Changed one of supportive analyses of the primary efficacy endpoint to an unadjusted logistic regression - Added robust estimation of unconditional treatment effect for the primary efficacy endpoint analysis	- The updates in primary analysis model as well as the addition of unconditional treatment effect were made to be aligned with the FDA draft guidance "Adjusting for Covariates in Randomized Clinical Trials for Drugs and Biological Products" dated in May 2021

TABLE OF CONTENTS

LIST OF TABLES

1 INTRODUCTION

This document provides the details of statistical analyses planned for Protocol No. NA-1-009/ ESCAPE-NEXT. In addition, it discusses the statistical issues relevant to these analyses (e.g., sample data to be used and missing data).

1.1 Background

Nerinetide (formerly termed NA-1 or TatNR2B9c) 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 nerinetide. The ability to identify patients with salvageable brain using the criteria used in the ESCAPE trial[2] provided an opportunity to target patients who may have the greatest benefit from neuroprotection, and to enhance further the impact of reperfusion therapies[3]. This led to the ESCAPE-NA-1 trial which used inclusion/exclusion criteria substantially similar to those of ESCAPE in order to select potential responders to neuroprotection by nerinetide. Results obtained from the ESCAPE-NA1 trial[4] suggested that nerinetide may be effective in enhancing functional independence, reducing mortality, and reducing infarct volumes in participants with AIS who were selected for EVT provided that they had not received alteplase, a thrombolytic agent indicated in some stroke patients. Our preclinical and clinical data support this notion.

The rationale for the present study is as follows:

1) The ESCAPE-NA1 trial (protocol NA-1-007) provided promising evidence that, in participants with AIS who were selected for EVT and who did not receive the thrombolytic alteplase, treatment with nerinetide increases functional independence and reduced stroke mortality[4]. This was supported by a reduction in infarction volume as measured by MRI or CT scanning. Since these improvements were observed in three separate domains of outcome (functional independence, mortality, and infarction volumes), they are unlikely to be due to chance alone. The present study is intended to explore these findings further.

2) Participants in ESCAPE-NA1 who did receive alteplase as part of their care did not benefit from subsequently being administered nerinetide. This was likely due to the degradation of nerinetide by circulating plasmin, the product of prior alteplase administration. This hypothesis was supported by pharmacokinetic data from a subset of participants in the trial that showed that those who received alteplase had reduced plasma levels of nerinetide (approximately 60% reduction) as compared to participants who did not receive alteplase. The magnitude of this reduction or its clinical impact could not have been anticipated from prior studies in animals.

3) Nerinetide does not have thrombolytic or thrombotic properties of its own[5] and does not interfere with the activity of thrombolytic agents such as alteplase or tenecteplase. It has a plasma half life of about 5-10 minutes after which it is redistributed to extravascular tissues. Because it is a peptide, it is cleaved by proteases. Preclinical studies have shown that plasmin, which has similar substrate specificity to trypsin, cleaves nerinetide into several fragments beginning at its N-terminus[5]. It is similarly cleaved when it is combined in plasma with alteplase or tenecteplase, which convert plasminogen in plasma into plasmin[5].

These same preclinical studies have shown that co-administration of nerinetide with high-dose alteplase (6x the human dose) can nullify the neuroprotective effectiveness of nerinetide in a rat model of embolic middle cerebral artery occlusion (eMCAO). However, due to its short half-life it can be administered in preclinical studies before alteplase and this restores its efficacy in eMCAO, and may be synergistic with alteplase[5]. The drug-drug interaction between nerinetide and alteplase has led to the exclusion of participants who are candidates for alteplase in the ESCAPE-NEXT trial.

4) Based on data from preclinical studies, the Phase 1 (protocol NA-1-001) safety study, the Phase 2 ENACT trial[6] (protocol NA-1-002) and the Phase 3 ESCAPE-NA1 study[4] (protocol NA-1-007), nerinetide is expected to be have an acceptable safety profile.
5) There is a compelling need to develop neuroprotectants in order to increase the proportion of patients who may benefit from EVT. These agents could improve the outcomes of patients and render more patients with AIS into candidates for endovascular or pharmacological recanalization treatment.
6) The current study is intended to confirm the findings in the ESCAPE-NA1 trial that nerinetide may improve functional independence, reduce mortality, and reduce infarction volumes in participants with AIS who are selected for EVT and who are not treated with thrombolytics.

1.2 Trial Objectives

1.2.1 Primary Objective

The primary objective is to determine the efficacy of the neuroprotectant, nerinetide, in reducing global disability in subjects with major acute ischemic stroke (AIS).

1.2.2 Secondary Objectives

The secondary objectives are to determine the efficacy of nerinetide in:

1) Reducing mortality rate
2) Reducing worsening of stroke*
3) Reducing functional dependence
4) Improving neurological outcome

* Worsening of stroke is defined as (A) progression, or hemorrhagic transformation, of the index stroke as documented by medical imaging that is (a) life-threatening requiring intervention and/or (b) results in increased disability as gauged by a ≥4 point increase from lowest NIHSS during hospitalization and/or (B) results in death from the index stroke.

1.2.3 Tertiary Objectives

The tertiary objectives are to determine the efficacy of nerinetide in:

- Decreasing infarct volume
- Improving activities of daily living
- Reducing dependency or death
- Improving excellent functional outcome. Improving health related quality of life

1.2.4 Safety Objectives

The 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 infusion of nerinetide to participant with acute stroke on SAEs and 90-day mortality.

1.2.5 1- Year Follow Up Analytic Sub-Trial Objectives

There will be a 1-Year follow-up analytic sub-trial to support the outcomes obtained at Day 90.

The primary objective is to determine the efficacy of the neuroprotectant, nerinetide in:

- Reducing global disability in participants with acute ischemic stroke (AIS).

The secondary objectives are to determine the efficacy of nerinetide in:

- Reducing mortality rate
- Improving activities of daily living
- Improving health related quality of life

Table 1: Objectives and Endpoints

Objectives	Endpoints*
Primary	
Reducing global disability in subjects with acute ischemic stroke (AIS).	The primary outcome is the proportion of participants with independent functioning on the modified Rankin Scale (mRS), as defined by a score of 0-2 at Day 90 post randomization. These participants are defined to be responders.
Secondary	
Reducing mortality rate.	A reduction in mortality rate, as defined by event rate (proportion, expressed as a percentage) for mortality over the 90-day study period.
Reducing worsening of stroke**.	Proportion of participants with worsening of stroke over the 90-day study period.
Reducing functional dependence.	A shift of one or more categories to reduced functional dependence analyzed across the whole distribution of outcomes on the mRS at Day 90 post randomization.
Improving neurological outcome.	Proportion of participants with good neurological outcome, as defined by a score of 0-2 on the NIHSS at Day 90 post randomization.
Tertiary/Exploratory	
Decreasing infarct volume.	Volume of stroke as measured by MRI or CT brain imaging (MRI preferred).
Improving activities of daily living.	Proportion of participants with functional independence in activities of daily living, as defined

	by a score of ≥ 95 on the Barthel Index (BI) at Day 90 post randomization.
Reducing dependency or death.	Proportion of participants with reduced moderate or severe disability or death, as defined by a score of 4-6 on the mRS at Day 90 post randomization.
Improving excellent functional outcome	Proportion of participants with excellent functional outcome, as defined by a score of 0-1 on the mRS at Day 90 post randomization
Improving health related quality of life.	Health-related quality of life, as measured by the EQ-5D-5L at Day 90.
Safety	
To determine the safety based on serious adverse events (SAEs).	Proportion of subjects with serious adverse events to Day 90.
90-day mortality.	Proportion of subjects alive at 90-day.
1 Year Follow Up	
Primary	
Reducing global disability in participants with acute ischemic stroke (AIS).	The proportion of participants with independent functioning on the modified Rankin Scale (mRS), as defined by a score of 0-2 at 1-year.
Secondary	
Reducing mortality rate	A reduction in mortality rate, as defined by event rate (%) for mortality over the 1-year study period.
Improving activities of daily living	The proportion of participants with independent function on activities of daily living defined on the modified Barthel Index (BI) with a score of ≥ 95 at 1-year.
Improving health related quality of life.	Health-related quality of life, as measured by the EQ-5D-5L at 1 year.

* Imputation for missing data up to Day 90 and for the 1-year follow up will be conducted as per Section 4.3 of this SAP.

**Worsening of stroke is defined as (A) progression, or hemorrhagic transformation, of the index stroke as documented by medical imaging that is (a) life-threatening requiring intervention and/or (b) results in increased disability as gauged by a ≥4 point increase from lowest NIHSS during hospitalization and/or (B) results in death from the index stroke.

1.3 Study Design

This study is a Phase 3, randomized, multicentre, blinded, placebo-controlled, parallel group, single-dose, adaptive design with a single interim analysis for unblinded sample size re-estimation. Because AIS is a medical emergency, the trial is designed to enable the administration of standard-of-care treatments without delay in order to save the life of the person concerned, restore good health or alleviate suffering.

Participants harboring an acute ischemic stroke who are selected for endovascular revascularization without intravenous or intra-arterial thrombolytic therapy will be given a single, 2.6 mg/kg (up to a maximum dose of 270 mg) intravenous dose of nerinetide or placebo. Randomization will be stratified by time from stroke onset to randomization ≤ 4.5 hours (yes/no) and done with stochastic minimization to balance baseline factors within strata. The end of the main trial is defined as the date that the last enrolled participant has completed their Day 90 visit/contact. For the purpose of an analytic follow-up sub-trial component, participants will be contacted by telemedicine or telephone at 1-year by individuals blinded to the outcome of the main trial.

An initial target 680, and up to 1020, male and female participants aged 18 years and older harboring AIS and who are selected for endovascular revascularization without intravenous or intra-arterial thrombolytic therapy will be enrolled. A patient who consents but is not randomized will be considered a screen failure. A subject is considered randomized the moment the real-time web-based randomization process is completed. This is time zero for the subject. 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 the pivotal study is defined as the date that the last enrolled subject has completed their Day 90 visit.

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).

Participants will be followed at 1-year for the analytic sub-trial for further outcome assessment by telemedicine or telephone interview conducted by individuals blinded to the outcome of the main trial. This sub-trial will be conducted to explore the independent functioning and quality of life at 1-year. This sub-trial will be reported separately from the main trial.

Subjects will be followed at 1-year for further outcome assessment by telephone interview conducted by individuals blinded to the outcome of the main study. The Schedule of Assessments for the main trial is presented in Table 2 and for the 1-Year follow up in Table 3.

Two database locks and corresponding reports are planned for this trial. The first report will be based on the completion of Day 90 visits for the main trial. The second report will be following the completion of the 1-Year follow up for the analytic sub-trial.

1.4 Sample Size Determination

The primary efficacy outcome variable for the pivotal assessment of efficacy is the overall proportion of participants experiencing a favorable functional outcome 90 days post-randomization, defined as a score of 0 to 2 on the modified Rankin Scale (mRS). These participants are defined to be responders.

An initial target 680, and up to 1020, male and female participants aged 18 or older harboring AIS and who are selected for endovascular revascularization without intravenous or intra-arterial thrombolytic therapy will be enrolled.

Based on results of ESCAPE-NA1 and assuming a 50% overall responder rate for the placebo group, there will be approximately 82% power to detect an 11.4% absolute effect difference between response rate (proportion of responders, with Day 90 mRS in the range 0 to 2) with nerinetide and placebo, at alpha level 0.05 2-sided.

This sample size was calculated via simulation under the assumption of 2% probability of dropout and 50 patients per month accrual during the trial (across both arms) using the Chen-DeMets-Lan approach[7] for unblinded sample size re-estimation, and possible early stopping for overwhelming superiority with an O'Brien-Fleming alpha spending boundary at a single interim analysis with a maximum sample size of 1020 evaluable (inflation factor 1.5) and inflation in the "promising zone" based on conditional power between 50% and 81%, based on the observed trend at the interim and using a Wald statistic with target conditional power after sample size increase 81% (using an increase rule to be specified in the IDMC Charter to prevent back-calculation of interim effect sizes after the interim analysis). The interim analysis is planned to take place at 75% information (primary endpoint), i.e. when approximately 510 of the initial target 680 patients have reached their primary endpoint assessment. With these specifications, the study will have approximately 82% power for the primary analysis using standard Wald statistic for comparison of 2 independent proportions (EaST v6.5 2020). The cumulative alpha spent at the interim analysis is 0.01 and final analysis 0.025, 1-sided; the stopping boundaries on the Z scale are 2.34 (interim) and 2.012 (final) and on the p-value scale 0.01 (interim) and 0.022 (final), all on the assumption that the interim is conducted at 75% information (EaST v6.5, 2020).

See Section 8 for further details on the interim analysis.

1.5 Randomization

Treatments (placebo vs. nerinetide) will be assigned 1:1 by application of a 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 as soon as possible after qualifying imaging and randomization. Although each vial of nerinetide or placebo will have a unique identification 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 enrolled at various times from stroke onset, treatment will be assigned using 1:1 randomization (nerinetide:placebo) with a stratification based on time from stroke onset to randomization of less than or equal to 4.5 hours (yes/no) and a randomized minimization algorithm to minimize the contribution of imbalances in baseline factors (age, sex, baseline NIHSS score, baseline ASPECT score, occlusion location, time from qualifying imaging to randomization, and site). The time of 4.5 hours was selected as it approximates the median time to randomization from the ESCAPE-NA1 trial.

There will be 2 randomization strata:

- Comprising subjects enrolled at a time of less than or equal to 4.5 hours from stroke

symptom onset.

- Comprising subjects enrolled at a time greater than 4.5 hours from stroke symptom onset.

Randomization will be by the minimally sufficient minimization procedure of Zhao[8]. The procedure will be implemented to achieve balance on 7 baseline subject-level prognostic variables: age, sex, baseline NIHSS scores, baseline ASPECT score, occlusion location, time from qualifying imaging to randomization and study site. At the time of randomization, subject age will be calculated using the central server date and time. This overall approach will ensure that the subjects entered into the trial will be matched on the seven key prognostic covariates, within strata and between treatment arms, thereby minimizing the likelihood of chance confounding 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 50 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 generated from the randomization website and will be transmitted to the central trial database to create the case in the electronic case report form. The randomization date, time, the stratification variable, and the 6 covariates for the minimization algorithm will not be editable once a subject is randomized.

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 from the local pharmacy will take place within one business day and as 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 2: Schedule of Activities- Main Trial

Visit/Contact	V1	V2	V3	V4	V5	V6
Day	**Day 1 Baseline**	**Day 1 Post-EVT**	**Day 2/3**	**Day 6[1] or discharge**	**Day 30[2]**	**Day 90[2]**
Window		**(~2 h)**	**(18-56 h)**		**(±5 d)**	**(-21 to +7d)**
Informed consent	X					
Regained capacity informed consent[3]			X	X	X	X
History and physical examination	X					
Weight[4]	X					
Vital Signs (BP, HR, Temperature) [5]	X	X	X			
Randomization/ Study drug administration	X					
Mortality		X	X	X	X	X
NIHSS	X	X	X	X	X	X
mRS[6]	X			X	X	X
Barthel Index	X				X	X
EQ-5D-5L						X
Qualifying Imaging	X					
Endovascular Procedure	X					
MRI/NCCT head[7]			X			
Laboratory Assessments	X[8]		X			
Pregnancy test[9]	X					
Pharmacokinetic samples[10]	X					
AE	Collected to Day 30					
SAE	Collected to Day 90					
Prior medications	X					
Concomitant medications	Collected to Day 6 or discharge					

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 a 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. 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.
5. Vital signs (BP, HR only) will be recorded immediately before and after completion of the study drug infusion, temperature will be collected at baseline only if standard of care.
6. Historical (pre-stroke) mRS score can be collected at any time.
7. MRI head may be supplanted by an NCCT head if MR is unavailable or contraindicated.
8. Blood should be drawn at baseline, but results are not required prior to randomization. Results from primary hospital (within 8 hours) are accepted.
9. If the participant 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.
10. PK samples will be collected from up to 100 participants: pre-dose and at 10, 20, 30 and 60 min after the start of study drug administration.

d = days; h = hours

Table 3: Schedule of Activities: One-Year Follow Up Telemedicine or Telephone Contact

Contact	V7- One-Year Follow up
Day (Window)	**Day 365 (±30 d)**
Mortality, EQ-5D-5L, mRS, Barthel Index	X

1.6 Blinding of Main Study

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 an unblinded statistician from a group that is independent of the sponsor and the blinded project team implementing the trial. A firewall will be maintained between the IDMC and statistician (unblinded) and the project staff (blinded). The IDMC will review safety data 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 if 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 study data management center to request unblinding of the specific subject. The data management center 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 study files.

1.7 Blinding of 1-year Follow-up Study

It is anticipated that a proportion of subjects enrolled in the Main Study will have reached their 1-year follow-up before the Main Study is unblinded. That proportion will depend on the rate of enrollment in the Main Study.

1.7.1 Subjects having the 1-year Follow-up While the Main Study is Blinded

Subjects whose 1-year follow-up occurs while the Main Study is still blinded will be contacted for telephone follow-up by study staff who are blinded to treatment allocation.

1.7.2 Subjects having the 1-year Follow-up After the Main Study is Unblinded

Subjects whose 1-year follow-up occurs after the Main Study is unblinded will be contacted for telephone follow-up by study staff who are firewalled from previous study staff who have become unblinded to treatment allocation. Specifically, such study staff will be firewalled from the Sponsor, the IDMC, the unblinded statistician, and the coordinating investigators.

1.8 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 24 hours prior to randomization. Concomitant medications are defined as those taken during study drug administration or after study drug has been administered through to Day 6 (or discharge). All prior and all concomitant medications will be recorded on the electronic case report form.

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.

Study Day: Day 1 is the date of randomization. As the study drug is intended to be administered as soon as possible after randomization (i.e., 15 minutes), this will also be the day of treatment initiation date. Study day is calculated relative to Day 1.

Treatment-Emergent Adverse Event: All AEs will be collected from the start of study drug administration until Day 30. All SAEs will be collected from the start of study drug administration until Day 90. A treatment-emergent adverse event (TEAE) is one that first occurs or worsens in severity or frequency after study drug administration has begun through to and including the Day 30. 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 the AE will be considered treatment-emergent.
- If the start date is completely missing then the AE will be considered treatment-emergent.

2 ANALYSIS POPULATIONS

This study is designed to address a population of adults 18 years and older harboring an acute ischemic stroke who are selected for endovascular revascularization without intravenous or intra-arterial thrombolytic therapy who will receive a single, 2.6 mg/kg (up to a maximum dose of 270 mg) intravenous dose of nerinetide or placebo.

2.1 This is a global trial being conducted under the same protocol at clinical sites in different countries in accordance with the national legislation of the country in which the trial is carried out. Following the completion of the study and provided that the requirements of 21CFR 312.120 are met, data from all study sites will be pooled for the purpose of analyses in this statistical analysis plan.Intent-to-Treat (ITT) 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 (PP) 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 the imaging inclusion criteria, 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 met the criteria.

Participants will be excluded from the per-protocol analysis for the following violations:

- did not meet inclusion/exclusion criteria,
- did not receive planned dose volume,
- incorrect study drug vial,
- infusion more than 15 minutes,
- consent not obtained (including incorrect Deferral of Consent where applicable).

3 STUDY ASSESSMENTS AND DATA COLLECTION

Study procedures and their timing are summarized in the Table 2: Schedule of Activities.

3.1 The Modified Rankin Scale

The primary endpoint used in this trial will be global disability, as measured by the mRS, at Day 90. The mRS is a valid and reliable clinician-reported measure of global disability that has been widely applied for evaluating recovery from stroke. It is a scale used to measure functional recovery (the degree of disability or dependence in daily activities) of people who have suffered a stroke[9,10]. mRS scores range from 0 to 6, with 0 indicating no residual symptoms; 5 indicating bedbound, requiring constant care; and 6 indicating death.

The post dose mRS will be obtained at Day 6 (or discharge), Day 30 and Day 90 and at the One-Year follow up. 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 (via www.healthcarepoint.com) in the use of this scale. An Electronic Clinical Outcome Assessment (eCOA) tool may be used to conduct this assessment.

3.2 Mortality

Mortality status will be obtained at all visits during the 90-day study period and at the One-Year follow up. Specifically, it will be assessed at Day 6 or discharge and at Days 30 and 90. Mortality rate is defined as the number of deaths observed divided by the number of subjects observed over the 90-day study period between nerinetide and placebo control subjects.

3.3 Worsening of Stroke

Worsening of stroke is defined as (A) progression, or hemorrhagic transformation, of the index stroke as documented by medical imaging that is (a) life-threatening requiring intervention and/or (b) results in increased disability as gauged by a ≥4 point increase from lowest NIHSS during hospitalization and/or (B) results in death from the index stroke.

3.4 National Institutes of Health Stroke Scale

The NIHSS is a standardized neurological examination score that is a valid and reliable measure of disability and recovery after acute stroke[11]. Scores range 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), Day 2, Day 6 (or discharge), Day 30 and Day 90. The NIHSS will only be scored by those trained in the use of this scale. An eCOA tool may be used to conduct this assessment. The NIHSS scores will be dichotomized into 0-2 (indicating a good neurological outcome) versus >2 (indicating otherwise).

3.5 Volume of Stroke

All subjects will undergo a follow-up brain MRI [including a minimum of axial DWI, gradient-echo (GRE), FLAIR] at Day 2 (18 to 56 hours from the time of randomization). The Day 2 MR is considered a standard of care imaging procedure; if MR is unavailable, then NCCT is allowed. The Day 2 MR (and where MR is unavailable, CT) will be used to assess infarct volume. Infarct volume determinations will be conducted before database lock.

Prior to database lock at 90 Days, the total volume of new strokes as measured by MRI or CT brain images (MRI preferred) in nerinetide versus placebo control participants will be calculated from the Day 2/3 imaging. Where MR is not available, infarct volumes will be determined from the Day 2/3 CT scan. The plan for combining CT and MRI data will be detailed in the Imaging Adjudication Charter.

3.6 Barthel Index

The BI is an index of functional independence[12] that is a valid measure of activities of daily living when employed in stroke trials[13]. Modified BI scores range 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), Day 30 and Day 90 and at the One-Year follow up, by those trained in the use of this scale. Note that the original Barthel Index was a scale from 0-20. The modified Barthel index simply multiplies the original scale by 5 to provide a 100-point score. An eCOA tool may be used to conduct this assessment.

3.7 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[14]. Each dimension has five response categories corresponding to: no problems, slight, moderate, severe and extreme problems[15]. The version of the instrument selected for the trial is interviewer administered either in-person, or by telemedicine or by telephone. The respondents will also rate their overall health on the day of the interview on a 0–100 visual analogue scale (EQ-VAS). The EQ-5D-5L will be administered at the Day 90 and at the One-Year follow up by those trained in the use of this scale. An eCOA tool may be used to conduct this assessment.

4 MISSING DATA AND DATA TRANSFORMATION

4.1 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; standard deviations 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.

The overall significance level for in this trial will be at two-sided 0.05. The type I error and the efficacy boundary for the final analysis will be adjusted accordingly based on the actual alpha spent at the interim analysis (details in Section 8).

In order to protect the overall trial false positive rate, the primary efficacy analysis and secondary outcome analyses 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 estimand
2) Secondary estimands, as specified in the order presented in Section 4.2.

4.2 Estimands

Death is an intercurrent event which may occur during the study. The fact that a participant has died may in itself be informative for quantifying the treatment effect, although data collections after death will not exist. Therefore, the primary and secondary estimands will be focusing on the composite strategy. More specifically, death will be incorporated as part of the primary and secondary estimands as described below and in Section 4.3.

The primary estimand will be the difference in the mRS response (i.e., mRS score of 0-2) proportions between treatment conditions (nerinetide vs. placebo) in the target patient population at Day 90. Deaths occurring over the Day 90 period will be considered as non-response.

The secondary estimands are defined below:

- The difference in mortality rate over the 90-day period between treatment conditions in the target patient population.
- The difference in worsening of stroke proportions between treatment conditions in the

target patient population over the 90-day period. Death meets the definition of worsening of stroke.

- The difference in showing a shift in mRS categories between treatment conditions in the target patient population at Day 90. Death occurring prior to Day 90 will be assigned with the worst mRS score (score of 6).
- The difference in NIHSS response (i.e., score of 0-2) proportions between treatment conditions in the target patient population at Day 90. Death occurring prior to Day 90 will be considered as non-response.

4.3 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.

All imputed values will be determined prior to database lock and conducting primary analyses via the rules documented prospectively in this Statistical Analysis Plan for the study.

To incorporate death as part of the estimand definition following the composite strategy, deceased participant will be assigned a score 6 on the mRS, 42 on the NIHSS and 0 on Barthel Index and be counted as non-responders. In addition, deceased participants are considered as experiencing worsening of stroke. Thus, there will be no missing mRS, NIHSS, BI or worsening of stroke data due to death.

No imputation is planned for safety data. Efficacy outcomes will be imputed using a data-informed imputation as follows:

4.3.1 Imputation of Mortality and Worsening of Stroke

For the analysis of rate of mortality, for participants for whom the mortality status is not known at Day 90:

- if they were alive at Day 30, then the subject will be imputed as alive at Day 90
- if both the Day 30 and Day 90 mortality is status is missing, the participant will be imputed as Dead at Day 90.

Worsening of stroke data will be imputed similarly:

- if it's unknown whether the subject experienced worsening of stroke by Day 90 while they did not report any worsening of stroke by Day 30, then the subject will be imputed as not experiencing worsening of stroke at Day 90.
- if both worsening of stroke status is unknown at both Day 30 and 90, then the subject will be imputed as experiencing worsening of stroke at Day 90.

No imputation will be done on the missing one-year follow-up mortality data.

4.3.2 Imputation of mRS Score

Deceased subjects will be assigned scores of 6 on the mRS.

Single Imputation (SI): To follow the composite strategy, the following approach will be used to impute missing mRS data at Day 90:

- if the participant is known to be dead at Day 90, they will be considered to be a non-responder and the mRS will be imputed as 6
- if the mRS was obtained at the Day 30 assessment or later and the participant is documented to be alive or the mortality status is unknown at Day 90, the Day 30 (or later) assessment will be carried forward as the Day 90 mRS value
- if both the Day 30 and Day 90 mRS scores are missing but the participant is documented to be alive at Day 90 they will be considered to be a non-responder and the mRS will be imputed as a 5
- if both the Day 30 and Day 90 mRS scores are missing and the mortality status of the participant is unknown at Day 90 they will be considered to be a non-responder and the mRS will be imputed as a 6.

If more than 5% subjects randomized are missing the mRS score at Day 90, additional imputation methods will be employed as follows:

Multiple Imputation (MI): The multiple imputation method assumes a Missing-At-Random (MAR) mechanism, which is a reasonable assumption for missingness not due to death. For the MI model, 50 imputations will be generated using PROC MI of SAS®. Imputation will be performed using the Fully Conditional Specification (FCS) logistic regression model. For each of the 50 datasets, missing mRS responders status (yes or no) will be imputed using a logistic regression model including treatment group, the mRS responder status at prior post-randomization visits, baseline mRS score, randomization stratification factor (time from stroke onset to randomization≤4.5 hours (yes/no)), age, sex, baseline NIHSS score, baseline ASPECT score, occlusion location, time from qualifying imaging to randomization. The seed to be used in all MI model is 20201009. The choice of 50 imputations is generally considered sufficient to provide accurate and stable imputed results given the amount of missingness expected in this trial[21].

Analysis of the primary outcome will then be performed for each of the relevant MI datasets. The results of the 50 analyses will be transformed into a normal statistic and combined into a single analysis using PROC MIANALYZE. Details see Section 6.1.3.

Example SAS code for PROC MI is as follows:

```
PROC MI DATA=ADMRS1 OUT=ADMRS2 SEED=20201009 NIMPUTE=50 NOPRINT;
     CLASS TRTP RANDTIME SEX BNIHSS BASPECT OCCLOC BASEMRS MRS2
     MRS4 MRS5 MRS6;
     FCS LOGISTIC(MRS4 = TRTP AGE RANDTIME SEX BASPECT OCCLOC
     BASEMRS / DETAILS);
     FCS LOGISTIC(MRS5 = TRTP AGE RANDTIME SEX BASPECT OCCLOC
     BASEMRS MRS4 / DETAILS);
     FCS LOGISTIC(MRS6 = TRTP AGE RANDTIME SEX BASPECT OCCLOC
     BASEMRS MRS4 MRS5 / DETAILS);
     VAR TRTP AGE SEX BNIHSS BASPECT OCCLOC RANDTIME IMA_TIME
     BASEMRS MRS2 MRS4 MRS5 MRS6;
RUN;
```

In above code, TRTP represents the randomized treatment group, BASEMRS = Baseline mRS score, MRS4 – MRS6 represents the binary mRS responder status at Day 6/discharge (Visit 4), Day 30 (Visit 5), Day 90 (Visit 6) respectively, BNIHSS = Baseline NIHSS score, BASPECT is the baseline ASPECT score (5-7 or 8-10), OCCLOC is the baseline occlusion location, RANDTIME is the time from stroke onset to randomization ≤ 4.5 hours (yes/no), IMA_TIME is the time from qualifying imaging to randomization.

The imputation models may be modified based on the actual data if there is an issue in model convergence.

Tipping Point Analysis: Tipping point analysis under missing not at random (MNAR) assumption will be performed as sensitivity analysis to assess the assumptions about the missing primary endpoint data. The tipping point analysis will apply a specified sequence of shift parameters that modify the imputed log odds of mRS response to non-response in the nerinetide group, as follows:

1. Missing mRS responder status will be imputed 50 times, following the MI method described above, with adjustment to the log odds of mRS response to non-response by shift parameter S1 in the nerinetide group only. A negative adjustment assumes that a subject with missing response status is more likely to be a non-responder; a positive adjustment assumes the subject with missing response status is more likely to be a responder. The MNAR statement with the ADJUST option in PROC MI will be used to apply the shift parameter.
2. Each of the 50 completed datasets applying the shift parameters will be analyzed using the primary analysis method as described in Section 6.1.3.
3. The results from the 50 completed datasets will be combined for inference using PROC MIANALYZE.
4. Repeat steps 1-3, with adjustment by a different shift parameter to the imputed log odds in the nerinetide group, as following: S1 = 0 (no shift), -0.2, -0.4, -0.6, -0.8, -1.0, -1.2, -1.4, -1.6, -1.8, and -2.0.

The shift parameters that result in a reversed study conclusion (i.e. from statistically significant to non-significant) will be flagged. Alternate series of shift parameters may be applied based on the actual data.

The missing mRS data at one-year follow-up will be handled according to below:

- If subjects are known to be dead before or at one-year follow-up visit, mRS will be assigned a score of 6 and counted as non-responders.
- Other missing data not due to death will be imputed by the multiple imputation method as described above.

4.3.3 Imputation of NIHSS and BI data

No missing NIHSS or BI data due to death. Deceased participants will be assigned scores 42 on the NIHSS and 0 on the Barthel Index (BI) and be counted as non-responders.

Missing NIHSS or BI data at Day 90 will be imputed using the last observation carried forward (LOCF) imputation as follows:

LOCF: Participants who are missing NIHSS or BI endpoint data at Day 90 will have the last

recorded score carried forward, provided that this score was obtained at the Day 30 visit or later. Otherwise, the missing NIHSS or BI will be imputed to the median score obtained at Day 90 in the trial.

For missing BI data at one-year follow-up visit:

- If the subjects are known to be dead before or at one-year follow-up visit, BI will be assigned a score of 0.
- Other missing BI data not due to death will be imputed to the median score obtained at one-year follow-up in the trial.

4.4 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 may be normalized with a cubic root transformation. No data transformation is planned for the primary analysis.

4.5 Pooling of Sites

In order to avoid sparse sites (sites with fewer than 10 randomized subjects) and the potential for too many levels for the explanatory variable of "site", sites within a geographic region (North America/Europe/Rest of World) with fewer than 10 randomized subjects will be pooled into a single pooled site for use in efficacy analyses. If a resulting pooled site still has fewer than 10 randomized subjects, it will be further pooled with the smallest remaining unpooled site(s) from that geographic region. If the resulting pooled site still has fewer than 10 randomized subjects, it will be further pooled with the smallest pooled site from another geographic region. The process of pooling sites will be reviewed and finalized before the unblinding of the main trial for the Day 90 analysis (i.e. first database lock as described in Section 1.3).

If more than one batch of nerinetide is used in the clinical trial, data from all subjects from all sites in the trial, regardless of the nerinetide batch administered, will be pooled for the purpose of analyses in this statistical analysis plan. Data from different batches will be pooled following the demonstration of equivalent quality of the clinical trial material based on the established release and stability testing of the nerinetide drug product batches used in the trial.

4.6 Pooling of Data from the Two Time-to-Randomization Strata

Since the randomization will be stratified by time from stroke onset to randomization ≤4.5 hours (yes/no), the assessment for treatment-by-stratification variable interaction to examine for effect modification will be performed via a log-binomial generalized linear model as described in Section 6.1.3. In addition, homogeneity of the randomization stratification factor will be tested via Breslow-Day statistic[22]. If there is no evidence for effect modification, then we will report the data for the entire cohort only. If there is evidence for effect modification, the effect size for the primary, secondary and tertiary outcomes will be additionally reported by stratum.

5 DISPOSTION AND DEMOGRAPHICS

5.1 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. A tabulation of the number and percentage of subjects randomized will be summarized by site for the ITT population.

Disposition will be listed by treatment arm and subject.

5.2 Protocol Violations

Protocol violations used to exclude subjects from the per-protocol analysis will be derived from the data collected in the case report form. Protocol violations will be determined during a data review meeting prior to database lock. Participants will be excluded from the per-protocol analysis for the following violations:

- Enrollment did not comply with inclusion/exclusion criteria
- Subjects who did not receive a per-protocol dose including: did not receive planned dose volume, received an incorrect study drug vial, or received the study drug infusion over more than 15 minutes.
- Consent not obtained (including incorrect Deferral of Consent where applicable).

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

5.3 Treatments

Per protocol, an intravenous solution of nerinetide 20mg/ml will be given to subjects with a body weight < 105 kg to achieve a final target dose of nerinetide 2.6 mg/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).

At the time of randomization, the dose will be calculated based on actual known weight (e.g., using an in-hospital scale) or on the subject's estimated weight. If the dosing weight was estimated, actual weight will be obtained by Day 5. Discrepancies between the weight estimate and actual weight obtained may result in some subjects not receiving the correct actual dose.

Site staff will record the total volume of nerinetide solution received by each subject and their weight at the time of dosing as well as the actual weight if an estimated weight was used. The following measures of the timeliness of the interventions and of exposure will be computed as follows.

1) Number and percentages of subjects who received any nerinetide
2) Descriptive statistics for:

- Time from stroke symptom onset to start of nerinetide infusion (minutes)

- Time from randomization to start of nerinetide infusion (minutes)
- Time from NCCT to start of nerinetide infusion (minutes)
- Duration of study drug infusion (minutes)
- Duration of study drug infusion (categorical): < 9 minutes, 9-11 minutes, > 11 minutes
- Actual administered volume of study drug (mL)
- Dosing compliance (%)
- Dosing compliance is calculated as actual administered volume of study drug divided by the baseline calculated volume of study drug * 100. The baseline calculated volume of study drug is the calculated volume based on the estimated or actual body weight at the time of randomization as recorded on the Case Report Form,
- Number and percentages of subjects compliant with study drug, a subject is considered compliant with study drug if the percent dosing compliance is ≥ 75% and ≤ 125% of planned volume.

All measures will be summarized for the ITT population; individual exposures will be listed by treatment arm and subject.

5.4 Study Progress Time

The following study progress time parameters will be summarized by treatment group and overall on ITT Population.

- Time from Stroke Onset to study drug infusion start (minutes)
- Time from ESCAPE-NEXT hospital arrival to study drug infusion start (minutes)
- Time from to study drug infusion start to initial reperfusion* (minutes)
- Time from ESCAPE-NEXT hospital admission to hospital discharge (days)
- Time in repatriation hospital (days)
- Time in inpatient rehabilitation (days)

*time of initial reperfusion is defined as the time logged for the first mTICI score.

5.5 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: age, sex, baseline NIHSS score, baseline ASPECT score, occlusion location, time from stroke symptom onset to randomization (hours), time from stroke symptom onset to randomization ≤4.5 hours (yes/no), time from qualifying imaging to randomization, unwitnessed stroke onset (yes/no), stroke-on-awakening (yes/no), and site. The summaries will be provided for the ITT, Per Protocol and Safety 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 stratification variable "Time from Stroke Onset to Randomization ≤ 4.5 hours" (yes/no)" in order to assess balance across treatment groups. Demographics will be listed by treatment arm and subject.

Relevant past medical history as well as prior and concomitant medications will be summarized and listed.

6 EFFICACY ANALYSIS

Two separate efficacy analyses are planned for this trial. The first analysis will be based on the completion of Day 90 visits for the main trial as described in Sections 6.1 to 6.5 below. The second analysis will be following the completion of the 1-Year follow up for the analytic sub-trial as described in Section 6.6 below.

A priori, we will assess for treatment-by-stratification variable interaction to examine for effect modification (details below). If there is no evidence for effect modification, then we will report the entire cohort only. If there is evidence for effect modification, we will additionally report results by stratum.

Efficacy analyses are summarized in Table 4: Summary of Inferential Efficacy Analyses.

6.1 Primary Outcome Variable Analysis for Main Study

6.1.1 Primary Estimand

The primary estimand is defined as the difference in the mRS response (i.e. mRS score of 0-2) proportions between treatment conditions (nerinetide vs. placebo) in the target patient population at Day 90. Death occurring prior to Day 90 will be considered as non-response.

6.1.2 Statistical Hypothesis

The primary hypothesis is:

$$H_0: \pi_{nerinetide} = \pi_{placebo} \quad VS \quad H_a: \pi_{nerinetide} \neq \pi_{placebo}$$

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

6.1.3 Primary Efficacy Estimand Analysis

Primary statistical analysis for primary estimand

The primary analysis for the primary estimand will be conducted in the ITT population according to the randomized treatment. Missing data will be imputed following the single imputation approach in Section 4.3.2.

The primary hypothesis to be tested is that administration of nerinetide will result in an increase in the proportion of mRS responders (as defined by a score of 0-2) at Day 90.

The pivotal primary analysis 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 pivotal main effects primary analysis will use a multivariable generalized linear model (GLM) using a binomial distribution with a log link to provide adjusted relative risk (log-binomial regression). Adjustment will include the stratification covariate of time from stroke onset to randomization ≤4.5 hours (yes/no) and the randomized minimization (age, sex, baseline NIHSS score, baseline ASPECT score, occlusion location, time from qualifying imaging to randomization and pooled site).

The main effects primary analysis will be supported by a further analysis in which the GLM used for the pivotal analysis also includes the interaction term (treatment by randomization stratification factor). If the interaction term is significant at the 0.05 level, we will additionally

repeat the primary analysis, sensitivity analyses, the supportive analyses described below, and the analyses for the secondary efficacy estimand analyses and tertiary efficacy outcome analyses for each stratum separately. If the interaction term is not significant at the 0.05 level, the data for each stratum will not be reported separately.

If a binomial distribution model fails to converge, accepted methods using multivariable logistic or Poisson regression with robust error variance will be used to provide adjusted relative risk.

Example SAS code for the log-binomial regression model as follows:

```
PROC GENMOD DATA = ADMRS;
     CLASS TRTPN RANDTIME SEX BASPECT OCCLOC SITEGR1;
     MODEL CRIT1FN(event="1") = TRTPN RANDTIME AGE SEX BNIHSS
     BASPECT OCCLOC IMA_TIME SITEGR1 / DIST = BINOMIAL LINK=LOG;
     ESTIMATE "NA-1 vs. placebo" TRTPN 1 -1 / exp;
RUN;
```

Example SAS code for the Poisson regression model:

```
PROC GENMOD DATA = ADMRS;
     CLASS TRTPN RANDTIME SEX BASPECT OCCLOC SITEGR1 USUBJID;
     MODEL CRIT1FN = TRTPN RANDTIME AGE SEX BNIHSS BASPECT
     OCCLOC IMA_TIME SITEGR1 / DIST = POISSON LINK=LOG;
     REPEAT SUBJECT=USUBJID / TYPE=IND;
     ESTIMATE "NA-1 vs. Placebo" TRTPN 1 -1 / exp;
RUN;
```

Where CRIT1FN is a numeric variable to indicate binary mRS responder status (1 = responder, 0 = non-responder), TRTPN is a numeric variable for treatment group (1 = nerinetide group, 2 = placebo), USUBJID is the subject ID.

The conditional treatment effect (i.e. relative risk) along with the 95% CI, as well as the corresponding Wald test statistics and p-value estimated from the above model will be reported. Actual proportions with and without independent functioning (i.e. mRS responders and non-responders) will be reported.

In addition, the unconditional treatment effect will be estimated and reported following below steps:

1. Run the primary analysis model (adjusted log-binomial regression) as described above.
2. For each subject, compute the model-based prediction of the probability of mRS response under nerinetide in both the nerinetide group and placebo group using each subject's specific baseline covariates (i.e. as specified in the primary analysis model)
3. Estimate the average response under nerinetide by averaging (across all subjects in the ITT population) the probabilities estimated in Step 2
4. For each subject, compute the model-based prediction of the probability of mRS response under placebo in both the nerinetide group and placebo group using each subject's specific baseline covariates

5. Estimate the average response under placebo by averaging (across all subjects in the ITT population) the probabilities estimated in Step 4
6. The estimates of average mRS responses rates in the two treatment groups from Steps 3 and 5 will be used to estimate the relative risk (i.e. unconditional treatment effect)

The 95% CI of the unconditional treatment effect will be constructed using a nonparametric bootstrap resampling, with 1000 resamples and subject as the unit of resampling. Resampling will be done independently for the nerinetide and placebo subjects. For each resample, Steps 1-6 will be repeated. The 95% CI of the unconditional treatment effect will then be determined from the distribution of resample relative risks.

Sensitivity analyses for the primary estimand

Sensitivity analyses in the ITT population will be conducted in which missing values are not imputed, i.e. observed cases (OC). In addition, the following analyses will be also be performed depending on the amount of missingness observed:

- If ≤ 5% of participants randomized are missing the Day 90 mRS assessment, a sensitivity analysis will be performed for the primary estimand by using only participants having completed Day 90 mRS assessments. Participants who are known to be dead before or at Day 90 will also be included as non-responders.
- If more than 5% participants randomized are missing the Day 90 mRS assessment:
    - a sensitivity analysis will be performed for the primary estimand with missing data imputed by MI (refer to Section 4.3.2)
    - A tipping point analysis will be performed to assess the robustness of the MAR assumption about the missing primary outcome

The sensitivity analysis performed on multiple imputed datasets will follow these steps:

1. For each of the multiple imputed datasets, the primary analysis model, i.e. adjusted log-binomial regression, will be performed. The log (relative risk) and the standard errors will be obtained.
2. The point estimate of the log (relative risk) and standard errors obtained from Step 1 will be passed to PROC MIANALYZE to generate a combined result. The combined log (relative risk), relative risk and associated 95% CI and the resulting p-value will be reported.

Supportive analyses for the primary estimand

Additional supportive analyses will be conducted in addition to the primary and sensitivity analysis to provide additional insights into the understanding of the treatment effect.

The primary analysis will be supported by a Cochran-Mantel-Haenszel test to evaluate the association between treatment and the primary outcome stratified by the randomization stratification variable (time from stroke onset to randomization ≤4.5 hours (yes/no). Homogeneity of the randomization stratification variable will be tested via Breslow-Day statistics. The common relative risk (nerinetide/placebo) over all strata with its 95% CI and p-value will be reported.

Example SAS code for the Cochran-Mantel-Haenszel test is as follows:

```
PROC FREQ DATA = ADMRS;
     TABLES RANDTIME*TRTP*CRIT1FL / CMH;
RUN;
```

A further supportive analysis will be an unadjusted two-sample comparison of binomial proportions via Wald test from logistic regression stratified on the randomization stratification factor (time from stroke onset to randomization ≤ 4.5 hours (yes/no)) to match the randomization. Estimated proportions (% responders) will be reported with binomial confidence intervals.

Example SAS code for the logistic regression test is as follows:

```
PROC LOGISTIC DATA = ADMRS;
     CLASS TRTP (PARAM=REF REF='Placebo');
     STRATA RANDTIME;
     MODEL CRIT1FL(EVENT="Y") = TRTP;
     ODDSRATIO TRTP / CL=WALD DIFF=ALL;
RUN;
```

Where CRIT1FL is the binary mRS responder status (yes/no).

Two additionally supportive analyses to the primary analysis will be conducted: (1) the primary analysis reapplied to the Per Protocol population with observed cases (OC) only; (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 nerinetide 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[2] and ESCAPE-NA1[4] trials.

Baseline, Day 6/Discharge, Day 30 and Day 90 mRS scores will be listed by treatment arm and subject.

6.2 Secondary Efficacy Estimand Analyses for Main Study

The primary analysis for the secondary estimands with binary endpoints will be based on the ITT population following the same methods as the primary estimand, and reported as described for each estimand including reporting by randomization strata if the interaction term in the GLM used for the primary pivotal analysis plus the interaction term is significant at the 0.05 level. Missing data will be imputed according to Section 4.3.

Binary secondary endpoints include: mortality rate, NIHSS responder and worsening of stroke.

For the secondary estimand of the "mRS shift analysis", the first step in the analysis will be an adjusted analysis with mRS score 5 and 6 combined, using a proportional odds model to derive the common odds of improvement ("shift") along the mRS scale. The mRS shift analysis will

only be conducted provided that the proportional odds assumption is found to be valid on testing. If it is found to be invalid, the remaining secondary endpoints will be deemed to be protected. Adjustment will include the same variables as the primary outcome analysis.

All tests will be conducted with two-sided level of significance alpha = 0.05 (overall for the in trial), adjusted for the interim analysis per the O'Brien-Fleming boundary spending function. 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. The secondary endpoints, and the order they are to be tested, are as they are listed in Table 1: Objectives and Endpoints.

Sensitivity analysis of the secondary estimands will be conducted in ITT population with missing data not imputed.

Additional analysis details of each outcome along with the supportive analyses of the secondary estimands are specified in each section below.

6.2.1 Mortality

Mortality rates, defined as the number of deaths observed divided by the number of participants observed over the 90-day study period between nerinetide and placebo control participants. Results will be summarized and tabulated.

In addition to the primary and sensitivity analysis, mortality analysis will be additionally supported using:

- Time-to-death survival function analysis, both unadjusted using Kaplan-Meier analysis and adjusted via Cox proportional hazards regression. Subjects who are known to be alive or the mortality status is unknown at study completion/discontinuation will be censored at the date of completion/discontinuation.
- Unadjusted logistic regression as described for the primary estimand

Mortality status scores will be listed by treatment arm and subject.

6.2.2 Worsening of Stroke

Worsening of Stroke is determined as the number of participants experiencing at least one worsening of stroke divided by the number of participants observed over the 90-day period in that treatment group, between nerinetide and placebo control participants. Results will be summarized and tabulated. The unadjusted logistic regression method will be used as a supportive analysis. The alpha protected secondary analysis of Worsening of Stroke as currently designed will also be supported by a further analysis in which the imaging requirement is waived, in order to include in this supportive analysis any cases in which the stroke worsened so rapidly that the patient was deemed palliative early on and did not undergo further imaging.

6.2.3 Secondary Outcome Analysis – mRS shift analysis

The primary 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 nerinetide 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% CI). 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. An adjusted POM will be used to derive the common odds of improvement (i.e. the nerinetide vs. placebo "shift" in mRS score distributions). Adjustment will include the same variables as the primary outcome analysis. The mRS shift analysis will only be conducted provided that the proportional odds assumption is found to be valid on testing. Deceased participants will be included with a mRS score of 6.

For the purpose of clarity, since the 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 outcomes will be deemed to be protected.

Example SAS code for POM testing as follows:

```
PROC LOGISTIC DATA=ADMRS;
     CLASS TRTP RANDTIME SEX BASPECT OCCLOC SITEGR1 / PARAM=GLM;
     MODEL AVAL = TRTP RANDTIME AGE SEX BNIHSS BASPECT OCCLOC
     IMA_TIME SITEGR1;
     EFFECTPLOT INTERACTION(X=TRTP SLICDBY=AVAL) / POLYBAR;
     ODDSRATIO TRTP / CL=WALD DIFF=ALL;
RUN;
```

Where "AVAL' is the collapsed mRS scale values ranging from 0 to 5 (level 5 = 5+6 mRS combined).

When SAS fits the POM, it runs a global test for a shift across all 6 mRS categories in the nerinetide 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:

1) The proportion of subjects with mRS = 0 vs. the proportion with mRS>0
2) The proportion of subjects with mRS ≤ 1 vs. the proportion with mRS>1
3) The proportion of subjects with mRS ≤2 vs. the proportion with mRS>2
4) The proportion of subjects with mRS ≤3 vs. the proportion with mRS>3
5) 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 a nerinetide-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)[16,17].

The results of the PO assumption tests, the common odds ratio estimate (with Wald 95% CIs) 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.

6.2.4 National Institutes of Health Stroke Scale

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 after day 30 in nerinetide versus placebo control subjects will be summarized and tabulated.

The same unadjusted logistic regression as discussed for the primary estimand will be used as a supportive analysis.

Baseline, Post EVT, Day 5, Day 30 and Day 90 NIHSS scores will be listed by treatment arm and subject.

6.3 Tertiary Efficacy Outcomes Analyses for Main Study

Summary statistics for each tertiary efficacy endpoint will be tabulated by treatment group. The tertiary analyses will be considered exploratory, and therefore are not subject to the fixed sequence multiple testing procedure to control the overall experiment-wise error rate for the trial as was done for the secondary analyses. These efficacy outcomes will be reported as described for each outcome including reporting by randomization strata if the interaction term in the GLM used for the primary pivotal analysis plus the interaction term is significant at the 0.05 level.

The tertiary efficacy endpoints include the:

- Volume of stroke as measured by MRI or CT brain imaging (MRI preferred).
- Proportion of participants with functional independence in activities of daily living, as defined by a score of ≥ 95 on the Barthel Index (BI) at Day 90 post randomization.
- Proportion of participants with reduced moderate or severe disability or death, as defined by a score of 4-6 on the mRS at Day 90 post randomization.
- Proportion of participants with excellent functional outcome, as defined by a score of 0-1 on the mRS at Day 90 post randomization.
- Health-related quality of life, as measured by the EQ-5D-5L at Day 90.

6.3.1 Volume of Stroke

Total volume will be assessed using an adjusted analysis comprising of a linear regression that includes the stratification and minimization variables, and supported by an unadjusted two-tailed Student’s t-test. A cubic root transformation will be performed if needed. Results will be reported with confidence intervals for the unadjusted and adjusted treatment effect between treatment groups.

Stroke volume will be listed by treatment arm and subject.

6.3.2 Barthel Index

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 nerinetide versus placebo control subjects will be analyzed using the same method as the primary analysis for the primary estimand. Results will be summarized and tabulated.

Baseline, Day 30 and Day 90 BI scores will be listed by treatment arm and subject.

6.3.3 Proportion of subjects with Day 90 mRS 4-6

The Day 90 mRS score will be dichotomized at mRS ≥ 4 (indicating moderate, severe disability or death) vs. mRS < 4 (indicating otherwise). The proportion of subjects with based on this dichotomy on Day 90 in nerinetide versus placebo control subjects will be analyzed as the primary analysis described for the primary estimand. Results will be summarized and tabulated.

6.3.4 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 nerinetide versus placebo control subjects will be analyzed using the same method as the primary analysis for the primary estimand. Results will be summarized and tabulated.

6.3.5 EQ-5D-5L

For the EQ-5D-5L, the difference between nerinetide 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 nerinetide/placebo treatment indicator variable and the stratification variables and covariates used in the supportive primary and secondary efficacy analyses. ANCOVA results will be summarized in a table. Least squared means, standard error and 95% CI will be reported.

The five dimensions of EQ-5D-5L (Mobility, Self-Care, Usual Activities, Pain/Discomfort, and Anxiety/Depression) will be summarized using frequency counts and percentages separately by treatment group.

Day 90 EQ-5D scores will listed by treatment arm and subject.

6.4 Exploratory Analyses for Main Study

In addition to the primary, secondary and tertiary analyses supported by the described adjusted analyses, exploratory subgroup analyses will be conducted to determine whether any of these factors can modify the effect of the nerinetide vs. placebo treatments. Sub-group analyses will be performed on the primary estimand as well as the secondary estimands that are not considered exploratory following the fixed sequence multiple testing procedure described in Section 6.2. In addition, forest plots will be generated to display effect sizes by sub-group[6]. They will be reported by randomization strata if the interaction term in the primary pivotal analysis plus the interaction term is significant at the 0.05 level.

Dichotomous sub-groups of interest include the following:

- Age (<80 years vs ≥ 80 years of age)
- Age (<65 years vs ≥ 65 years of age)
- Sex (men vs. women)
- Ethnicity (Hispanics vs non-Hispanic)
- Race (White, Asian, Black, Other)
- Baseline stroke severity (NIHSS > Median vs. NIHSS <= Median)
- Baseline occlusion location (MCA [including M1 and M2] vs. ICA)
- Baseline ASPECT score (5-7 vs. 8-10)
- Time from qualifying imaging to study drug initiation (greater than vs. less than the median).
- Time from onset of stroke symptoms to start of study drug greater than vs. less than the median).
- Degree of reperfusion (TICI ³ 2b vs. TICI <2b)
- Subjects weighing between 105-120 kg.

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/secondary endpoints, 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 (nerinetide/placebo) odds ratios, with 95% confidence intervals will be the nerinetide effect size estimates for each of the subgroups (e.g., for males and for females) and will be displayed in the forest plots.

6.5 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 nerinetide 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 nerinetide will be excluded from analysis (placebo).

Descriptive statistics will be calculated for nerinetide plasma concentrations and for all PK

parameters (AUC_{0-t}, $AUC_{0-\infty}$, C_{max}, T_{max}, $t_{½}$).

PK results at all timepoints will be listed for the Nerinetide arm by subject.

6.6 Outcome Analysis for 1-Year Sub-Study

The outcomes of the 1-Year Sub-Study are intended to be supportive of the 90-day outcomes of the main study. The analysis population will be comprised of participants with a valid consent for the 1-year sub-study in the relevant jurisdiction. Missing data will be imputed as per Section 4.3 except that for subjects who are successfully contacted (i.e., not deceased) but who cannot complete the telephone interview (e.g., due to dysphasia, a language barrier, or incapacity) will have their outcomes estimated by the interviewer. A summary table will specify the differences between the population analyzed in the main study and the 1-year sub-study, including deaths, losses to follow-up and withdrawal of consent.

The primary outcome is the proportion of subjects with independent functioning on the modified Rankin Scale (mRS), as defined by a score of 0-2) at 1 year.

The secondary outcomes include:

- A reduction in mortality rate, as defined by event rate (%) for mortality over the 1-year study period.
- The proportion of subjects with independent function on activities of daily living defined on the modified Barthel Index (BI) with a score of ≥ 95 at 1 year.
- Health-related quality of life, as measured by the EQ-5D-5L at 1 year.

6.6.1 mRS at 1 Year

The mRS will be assessed as per the protocol. It will be dichotomized, analyzed and reported as in the primary efficacy analysis.

6.6.2 Mortality Rate at 1 Year

Mortality at 1 year will be assessed as per the protocol (from family or legally authorized representative or from hospital records). It will be analyzed and reported as described in Section 6.2.1.

6.6.3 Barthel Index at 1 Year

The Barthel Index will be obtained as per the protocol from trial participants at 1 year. It will be dichotomized, analyzed and reported as for the primary analysis.

6.6.4 EQ-5D-5L at 1 Year

The EQ-5D-5L will be obtained by telephone or in-person interview from trial participants at 1 year. EQ-5D-VAS will be analyzed and reported as described in Section 6.3.5.

Table 4: Summary of Inferential Efficacy Analyses

Endpoint		Primary Analysis*	Sensitivity Analysis*	Supportive Analysis*
Primary	Day-90 mRS Responder (≤2 vs. >2)	Adjusted log-binomial regression model, ITT, SI Estimation of unconditional treatment effect will also be reported.	- Adjusted log-binomial regression model, ITT, OC - Adjusted log-binomial regression model, ITT with Day 90 completers only (if missingness ≤ 5%) - Adjusted log-binomial regression model, ITT, MI (if missingness > 5%) - Adjusted log-binomial regression model, ITT, Tipping Point Analysis (if missingness > 5%)	- Primary analysis with the interaction term added (treatment by randomization stratification factor) - Adjusted log-binomial regression model, ITT, Re-randomization - CMH, ITT, SI - Unadjusted logistic regression, ITT, SI - Adjusted log-binomial regression model, PP, OC
Secondary	Day-90 Mortality	Adjusted log-binomial regression model, ITT Missing data imputed based on Day 30 status	Adjusted log-binomial regression model, ITT, OC	Mortality Rate: - Unadjusted logistic regression, ITT, Missing data imputed based on Day 30 status - Adjusted log-binomial regression model, PP, OC Time to Death (Days): - Kaplan Meier, ITT, OC - Cox proportional hazard regression, ITT, OC
	Worsening of Stroke	Adjusted log-binomial regression model, ITT, Missing data imputed based on Day 30 status	Adjusted log-binomial regression model, ITT, OC	- Unadjusted logistic regression, ITT, Missing data imputed based on Day 30 status - Adjusted log-binomial regression model PP, OC
	Day-90 mRS shift (Ordinal)	POM, ITT, SI	- POM, ITT, OC - POM, ITT with Day 90 completers only	POM, PP, OC
	Day-90 NIHSS Responder (≤2 vs. >2)	Adjusted log-binomial regression model, ITT, LOCF	Adjusted log-binomial regression model, ITT, OC	- Unadjusted logistics regression, ITT, LOCF - Adjusted log-binomial regression model, PP, OC
Tertiary	Stroke volume	Linear regression, ITT, OC	N/A	Student's t-test, ITT, OC
	Day-90 Barthel Responder (<95 vs.≥95)	Adjusted log-binomial regression model, ITT, OC	N/A	N/A
	Day-90 mRS	Adjusted log-binomial	N/A	N/A

Endpoint		Primary Analysis*	Sensitivity Analysis*	Supportive Analysis*
	Responder (≤3 vs. ≥4)	regression model, ITT, SI		
	Day-90 mRS Responder (≤1 vs. >1)	Adjusted log-binomial regression model, ITT, SI	N/A	N/A
	Day 90 EQ-VAS	ANCOVA, ITT, OC	N/A	N/A
1 Year	mRS Responder (≤2 vs. >2)	Adjusted log-binomial regression model, ITT, MI	N/A	N/A
	Day-90 Mortality	Adjusted log-binomial regression model, ITT, OC	N/A	N/A
	Barthel Index	Adjusted log-binomial regression model, ITT	N/A	N/A
	EQ-VAS	ANCOVA, ITT, OC	N/A	N/A

*For analyses using the adjusted log-binomial regression model, a Poisson model with robust variance will be used if the log-binomial model fails to converge

7 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.

7.1 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 nerinetide 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.

A listing of Adverse Events of Special Interest (AESIs) can be found in Appendix 9.1

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, Possibly, Unrelated).

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

- Overview of Adverse Events
- TEAEs (with a start date 0-30 days) by MedDRA SOC and 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 nerinetide 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, Possibly, Unrelated) by MedDRA SOC and by preferred term. Missing relationships will be assumed as ‘related’.

- Serious TEAEs (with start date 0-90 days) by relationship to study treatment (Related, 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
- TEAESIs (onset 0-120 minutes post end of study drug infusion and of special interest as defined in Appendix 1: Listing of Treatment-Emergent AEs of Special Interest (TEAESI)) 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 TEAEs leading to death by MedDRA SOC and by preferred term
- All SAEs related to study drug by MedDRA SOC and by preferred term "Related" will include Related, Possibly and missing relationship
- All TEAESIs by MedDRA SOC and by preferred term
- All TEAEs resulting in discontinuation of treatment

7.2 Vital Signs

A summary (table) of blood pressure (systolic and diastolic) will be reported at Baseline/Visit 1, immediately pre dose (Visit 1), post dose (Visit 1), Visit 2 (Post EVT) and at Visit 3 (Day 2/3). Absolute values and changes from Baseline (Visit 1) to post dose (Visit 1), Visit 2 (Post EVT) and at Visit 3 (Day 2/3) will be summarized descriptively.

A summary (table) of heart rate will be reported at Baseline/Visit 1 (pre-dose), Visit 2 (Post EVT) and at Visit 3 (Day 2/3). Absolute values and changes from pre-dose to Post EVT and Day 2 will be summarized descriptively.

A summary (table) of temperature will be reported Baseline/Visit 1 (pre-dose), Visit 2 (Post EVT) and at Visit 3 (Day 2/3). Absolute values and changes from pre-dose to Post EVT and Day 2 will be summarized descriptively.

A listing of all vital signs will be provided.

7.3 Laboratory Results

A summary (table) of complete blood count (Platelets, hematocrit and hemoglobin), electrolytes (sodium, potassium and chloride) and chemistry (serum creatinine and serum glucose) will be reported at Baseline and Day 2.

Absolute values and change from baseline values for laboratory results will be summarized descriptively. Inferential statistics (ie, p-values or CI) will not be provided for these data.

A listing of all laboratory results as well as abnormal lab values post-dose will be provided.

7.4 Concomitant Medications

All concomitant medications collected on the Case Report Form with a start date prior to the date of Visit 4 (Visit will occur at Day 6 or hospital discharge if prior to Day 6) will be summarized by ATC Level 1 and Preferred Term within treatment group as well as listed in by-treatment by-subject listings.

8 INTERIM ANALYSIS

There will be an unblinded interim analysis and sample size re-estimation (SSRE) conducted in this trial when approximately 510 participants (75% information based on initial sample size) completed their 90-day study visit (that is when 510 participants have either completed Day 90 visit or discontinued from the study prior to Day 90 visit).

The interim analysis will be conducted by the unblinded statistician in the independent Statistical Group. An IDMC will review the interim efficacy and SSRE results.

Depending on enrollment rates, it may be necessary to perform the interim analysis before 75% information to ensure sample size re-estimation is completed before the study has been fully enrolled.

8.1 Interim Efficacy Analysis

There will be an interim efficacy analysis for unblinded sample size re-estimation in this trial. It will be conducted by the unblinded statistician in the Independent Statistical Group.

The interim efficacy analysis will be performed after approximately 510 participants have complete the Day 90 follow-up, at 75% information on the primary endpoint. The interim efficacy analysis will be conducted on the ITT population, following the same methods as the study final analysis described in Table 5 below.

Table 5: Summary of Interim Efficacy Analyses

Endpoint		Analysis
Primary	Day-90 mRS Responder (≤2 vs. >2)	Adjusted log-binomial regression model, ITT, SI
Secondary	Day-90 Mortality	Adjusted log-binomial regression model, ITT Missing data imputed based on Day 30 status
	Worsening of Stroke	Adjusted log-binomial regression model, ITT, Missing data imputed based on Day 30 status
	Day-90 mRS shift (Ordinal)	POM, ITT, SI
	Day-90 NIHSS Responder (≤2 vs. >2)	Adjusted log-binomial regression model, ITT, LOCF

The planned initial sample size is of 680 evaluable participants randomized 1:1, allowing for a single interim analysis at 75% information (when about 510 patients have primary endpoint assessments) with O'Brien-Fleming alpha-spending function stopping boundary for overwhelming efficacy as well as the possibility of sample size re-estimation based on conditional power at the same interim look using the Chen-DeMets-Lan approach[7] for unblinded sample size re-estimation. The trial-wise alpha (FWER) will be controlled at pivotal, 1-sided 0.025 level via primary analysis using standard group sequential Wald test for comparison of 2 independent proportions[7,18] (EaST v6.5 2020), in spite of the sample size re-estimation, by the "promising zone" method using the Chen-DeMets-Lan approach[1.] The cumulative alpha spent at the interim analysis is 0.01 and final analysis 0.025, 1-sided; the stopping boundaries on the Z scale are 2.34 (interim) and 2.012 (final) and on the p-value scale 0.01 (interim) and 0.022 (final), all on the assumption that the interim is conducted at 75% information (EaST v6.5, 2020). If the interim analysis is conducted at a different time point other than when 75%

information available, the type I error and the efficacy boundary for the interim and final analysis will be adjusted accordingly using nQuery Advisor®.

Power calculations for the unblinded sample size re-estimation design were run via simulation under the assumption of 2% probability of dropout during the trial (across both arms) using the Chen-DeMets-Lan approach[1] for unblinded sample size re-estimation with a maximum sample size of 1020 randomized (sample size re-estimation inflation factor 1.5) and inflation based on conditional power promising zone between 50% and 80% based on the observed trend at the interim and using a Wald statistic (using a sample size increase rule to be specified in the IDMC Charter to prevent back-calculation of interim effect sizes).

The IDMC may recommend stopping for overwhelming efficacy at the interim analysis if the test statistic crosses the O-F boundary. Additionally, the IDMC may recommend a sample size modification for the trial per the pre-specified re-estimation criteria which are outlined in the IDMC Charter, based on the interim results provided by the Independent Reporting Statistician/Independent Statistical Center to the IDMC. However, notwithstanding the O-F superiority critical p-value of 0.01 for stopping (boundary value) at the interim analysis of the primary efficacy outcome, the IDMC will be instructed to make a recommendation on stopping per their own review of all data, and also, in support of their recommendation, to report to the Sponsor/Steering Committee whether or not the superiority boundary was crossed and how the study sample size should be adjusted after the interim analyses. The IDMC Charter will provide further details on the rationale for, and how, these recommendations will be communicated.

The following summary tables and analyses will be performed at the interim analysis (also see Table 5) and provided to the IDMC in support of consideration of stopping for overwhelming efficacy at the interim analysis:

- Subject disposition (e.g., number completed Day 90 visits) by treatment group
- Subject demographics and baseline characteristics (as described in Section 5.5) by treatment group
- Primary efficacy endpoint:
    - Proportion of participants with independent functioning on the modified Rankin Scale (mRS), as defined by a score of 0-2 (mRS responder) at Day 90
- Secondary efficacy endpoints:
    - Mortality rate over the 90-day study period
    - Proportion of participants with worsening of stroke over the 90-day study period
    - Shift in mRS categories at Day 90 (mRS shift analysis)
    - Proportion of participants with a score of 0-2 on the NIHSS at Day 90

By-subject data listings will also be provided to support the interim tables.

The unblinded statistician in the Independent Statistical Group will also perform the conditional power calculation and sample size re-estimation by using nQuery Advisor® (Interim Monitoring and Unblinded Sample Size Re-estimation Module). The sample size increase rules will be provided to the unblinded statistician directly by the external regulatory statistician as per IDMC Charter, and will not be shared with any blinded study team members prior to database lock. The nQuery results, including the Wald test statistics and conditional power based on the treatment

effects at interim, boundary values per correct information time of interim analysis, sample size re-estimation rules, the re-estimated new sample size (if applicable) and the conditional power based on the new sample size will be provided to IDMC in a format (e.g. executive summary in a report or presentation slides) as deemed appropriate by the unblinded statistician.

8.2 Safety Analysis

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, and discontinuations due to AEs. The IDMC may recommend stopping for safety at the interim analysis.

The following safety summaries (tables) will be provided to the IDMC:

- Overview of Adverse Events
- TEAEs (with a start date 0-30 days) by MedDRA SOC and 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
- All TEAEs resulting in discontinuation of treatment, 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

8.3 Independent Data Monitoring Committee

Activities, mandate, responsibilities, communication structure and function of the IDMC will be documented in the IDMC Charter prospectively. This will also include a Blinding Plan specifying sequestering and blinding measures planned for the trial (including analysis firewalls) to prevent operational bias from revelation outside the IDMC of any aggregate interim results on safety or efficacy by treatment arm.

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. The IDMC Charter (separate document) captures details of the firewalls.

The unblinded Independent Statistical Group will be sequestered from the Project Team, steering committee and investigators and 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.

The Independent Statistical Group is responsible to:

- Prepare Tables, Figures and Listings for the IDMC to review
- Prepare interim conditional power results and uSSR information for the IDMC to review (per nQuery, see Section 8.1above)
- 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, conditional power results and uSSR information to the IDMC with an Interim Report (or presentation slides with executive summary) for the Interim uSSR

The Global Coordinating Investigator will handle all blinded communication with the IDMC members. The IDMC Independent Reporting Statistician, also a member of the Independent Statistical Group, also attends the Open and Closed Sessions of the IDMC meetings and answers any questions from the IDMC regarding the reports. The IDMC Chair will take minutes for the closed sessions.

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.

8.4 Handling of Enrollment Overage

The interim analysis is anticipated to take place once 510 participants have reached their 90 day follow-up. The IDMC may recommend to (1) stop the trial due to overwhelming efficacy (2) to complete enrollment to the originally-planned 680 participants or (3) to increase the sample size based on the results of the sample-size re-estimation up to 1020 participants.

It is possible that, by the time the IDMC provides a recommendation, enrollment in the study could exceed the number of participants needed to adhere to the IDMC recommendation. In such an eventuality, the primary outcome variable analysis for the main study and for the 1-Year Sub Study, as well as the Secondary Efficacy Estimand Analyses for the Main Study and for the 1-Year Sub Study may also be performed on the participants as per the IDMC-recommended sample size as needed.

9 SUPPORTING DOCUMENTATION

9.1 Appendix 1: Listing of Treatment-Emergent AEs of Special Interest (TEAESI)

Table A-1: AEs Related to Angioedema (by preferred term) 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 preferred term) 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 preferred term) 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

9.2 Appendix 2: List of Abbreviations

AE	Adverse Event
AESI	Adverse Events of Special Interest
AIS	Acute Ischemic Stroke
ANCOVA	Analysis of Covariance
ASPECTS	Alberta Stroke Program Early Computerized Tomography Score
ATC	Anatomical Therapeutic Chemical
AUC	Area under the Curve
BI	Barthel Index
BP	Blood Pressure
CT	Computerized Tomography
CTA	Computerized Tomographic Angiography
CTP	Computerized Tomographic Perfusion
DWI	Diffusion Weighted Imaging
eCOA	electronic Clinical Outcome Assessment
eMCAO	Embolic Middle Cerebral Artery Occlusion
EQ-5D-5L	EuroQol 5-dimension 5-level (quality of life)
EVT	Endovascular Thrombectomy
FLAIR	Fluid Attenuated Inversion Recovery
GRE	Gradient Echo
ICA	Internal Carotid Artery
IDMC	Independent Data Monitoring Committee
IND	Investigational New Drug (USA)
ITT	Intent-to-Treat
IV	Intravenous
LOCF	Last Observation Carried Forward
MedDRA	Medical Dictionary for Regulatory Activities
MCA	Middle Cerebral Artery
mCTA	Multiphase Computerized Tomographic Angiography
MR	Magnetic Resonance
MRI	Magnetic Resonance Imaging
MRP	Magnetic Resonance Perfusion
mRS	Modified Rankin Scale
NCCT	Non-contrast Computed Tomography Scan
NIHSS	National Institutes of Health Stroke Scale
OC	Observed Cases
O-F	O'Brien-Fleming
OR	Odds Ratio
PK	Pharmacokinetic
PO	Proportional Odds

POM	Proportional Odds Model
PP	Per Protocol
PSD-95	Post-Synaptic Density 95
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SI	Single Imputation
SOC	System Organ Class
SSRE	Sample Size Re-Estimation
TEAE	Treatment-emergent Adverse Event
TEAESI	Treatment-emergent Adverse Events of Special Interest
TICI	Thrombolysis in Cerebral Infarction
VAS	Visual Analogue Scale
wt	Weight

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