1.0 TITLE PAGE

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)
Investigational Product:	Nerinetide
Indication Studied:	Acute Ischemic Stroke
Name of Sponsor:	NoNO Inc.
Protocol Number:	ESCAPE-NEXT (NA-1-009)
Trial Registry Number(s):	clinicaltrials.gov number NCT04462536 EudraCT number 2020-002360-30
Development Phase:	Phase 3
Study initiation date (first participant enrolled):	06 December 2020
Study completion date (last participant completed):	28 April 2023 (to Day 90 visit) 16 June 2023 (One year follow up)
Name and affiliation of the Coordinating Investigator:	Michael D. Hill M.D., MSc, F.R.C.P.C. Director, Calgary Stroke Unit University of Calgary Calgary, Alberta, Canada
Name of Sponsor Signatory:	Michael Tymianski, M.D., Ph.D., F.R.C.S.C. NoNO Inc. 333 Bay Street, Suite 2400 Toronto, Ontario, Canada M5H 2T6
Date of Report	Final (Primary Use) Report: 19 Apr 2024

GCP Compliance: The trial was performed in compliance with International Council for Harmonisation and Good Clinical Practices guidelines, including the archiving of essential documents as well as the ethical principles of the Declaration of Helsinki.

2.0 SYNOPSIS

Name of Sponsor/Company: NoNO Inc.	(For National Authority Use only)
Name of Finished Product: Nerinetide (NA-1, 2.6 mg/kg)	
Name of Active Ingredient: NA-1	

Title of Trial:	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 Number:	ESCAPE-NEXT (NA-1-009)	
Investigators and Study Centers:	Coordinating Investigators: Michael Hill M.D., Calgary, Canada, Mayank Goyal M.D., Calgary, Canada 77 investigators at 77 sites in Canada, United States of America, Germany, Italy, Netherlands, Norway, Switzerland Australia and Singapore.	
Publication (reference):	Pending at the time of this report	
Studied period (years):	First Subject Enrolled: 06 December 2020 Last Subject Enrolled: 31 January 2023 Last Subject Visit (Day 90): 28 April 2023 Last Subject Visit (One Year- Early Termination): 16 June 2023	**Phase of Development:** 3

Trial Rationale:

The rationale for the ESCAPE-NEXT trial was based on promising results from the recently completed ESCAPE-NA1 trial and was intended to confirm the findings that nerinetide could improve functional independence, reduce mortality, and reduce infarction volumes in participants with AIS who were selected for endovascular thrombectomy (EVT) and who were not treated with thrombolytics.

ESCAPE-NA1 was a randomized, multicentre, blinded, placebo-controlled, parallel group, single-dose trial. 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 was consistent with the hypothesis that nerinetide was cleaved and inactivated as a result of the administration of alteplase. The data from ESCAPE-NA1 were also consistent with a significant body of preclinical studies in rodents and primates, which showed that nerinetide reduces infarct burdens and improves functional outcomes in experimental animals. The effectiveness of nerinetide in participants who did not receive prior alteplase provided the rationale for selecting such participants for ESCAPE-NEXT.

Name of Sponsor/Company: NoNO Inc.	(For National Authority Use only)
Name of Finished Product: Nerinetide (NA-1, 2.6 mg/kg)	
Name of Active Ingredient: NA-1	

There is a compelling need to develop neuroprotectants in order to increase the proportion of patients who could 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.

Trial design:
Because acute ischemic stroke (AIS) is a medical emergency, the trial was 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.

Trial population:

In order to evaluate neuroprotection in acute ischemic stroke patients who are selected for endovascular therapy were enrolled into the trial.

Treatment:
The 2.6 mg/kg dose was chosen for this clinical trial because of (1) the safety profile observed in the previous Phase 1 and 2 clinical trials, (2) the observed capacity of this dose of NA-1 to reduce stroke tissue damage and to improve neurological function in rats and non-human primates exposed to experimental strokes when NA-1 was administered in animals exhibiting salvageable brain, 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.

Objectives and Outcomes-Main Trial (Day 90)

Objectives	Outcomes
Primary	
To determine the efficacy of the neuroprotectant nerinetide in 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 post randomization.
Secondary	
To determine the efficacy of nerinetide in:	
• 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

Name of Sponsor/Company: NoNO Inc.	(For National Authority Use only)
Name of Finished Product: Nerinetide (NA-1, 2.6 mg/kg)	
Name of Active Ingredient: NA-1	

	distribution of outcomes on the mRS at Day 90 post randomization.
• Improving neurological outcome.	• Proportion of participants good neurological outcome, as defined by a score of 0-2 on the NIHSS at Day 90 post randomization.
Tertiary/Exploratory	
To determine the efficacy of nerinetide in:	
• 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 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 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 Serious adverse events and 90-day mortality:	• Incidence of serious adverse events and 90-day mortality.

Abbreviations: CT=computed tomography; MRI=magnetic resonance imaging, NIHSS=National Institutes of Health Stroke Score, EQ-5D-5L=Euro Quality of Life 5 Dimension 5 Level.

Objectives and Outcomes for the 1-Year Follow-up (not included in this report)

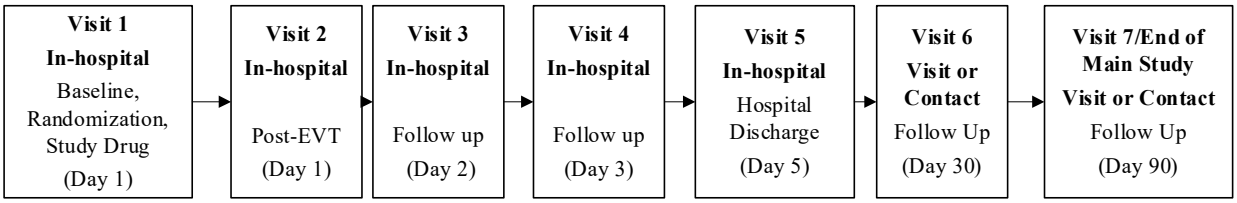
Objectives	Outcomes
Primary	
To determine the efficacy of the neuroprotectant, nerinetide in reducing global disability in participants with acute ischemic stroke.	The proportion of participants with independent functioning on the modified Rankin Scale, as defined by a score of 0-2 at 1-Year.

Name of Sponsor/Company: NoNO Inc.	(For National Authority Use only)
Name of Finished Product: Nerinetide (NA-1, 2.6 mg/kg)	
Name of Active Ingredient: NA-1	

Secondary	
To determine the efficacy of nerinetide in: 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.	• Proportion of participants with independent function on activities of daily living defined on the modified Barthel Index 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.

Methodology:

ESCAPE-NEXT was a Phase 3, multicenter, randomized, multicentre, blinded, placebo-controlled, parallel group, single-dose design with a single interim analysis for safety and efficacy.



Participants Identification and Treatment

Participants were identified using usual standard of care screening methods at the acute stroke hospital. This included screening by neurology residents, fellows, nurse practitioners, physician assistants or faculty physicians. Sites were selected if they have established mechanisms for screening this population of participant. This includes standard of care use of NCCT and CTA.

A single dose of nerinetide or matching placebo volume was administered as a 10 minute IV infusion on Day 1.

Post-Treatment Follow-Up Period

All participants in the main trial were followed for 90 days (or until death if prior to 90 days). The end of the main trial was defined as the date that the last enrolled participant completed their Day 90 visit/contact.

A subgroup of trial participants were followed to 1-Year.

Name of Sponsor/Company: NoNO Inc.	(For National Authority Use only)
Name of Finished Product: Nerinetide (NA-1, 2.6 mg/kg)	
Name of Active Ingredient: NA-1	

Number of participants (planned and analyzed): 850 male and female participants harboring AIS and who were selected for endovascular revascularization without IV or intra-arterial thrombolytic therapy were enrolled.

Diagnosis and main criteria for inclusion:

The eligibility of male and female patients to be enrolled in the trial was determined based on the following inclusion/exclusion criteria:

Main Inclusion Criteria

- Acute ischemic stroke (AIS) selected for emergency endovascular treatment.
- Age 18 years or greater.
- Onset (last-known-well) time to randomization time within 12 hours.
- Disabling stroke defined as a baseline NIHSS > 5 for internal carotid artery (ICA) and M1-middle cerebral artery (MCA) occlusion; or NIHSS > 10 for M2-MCA occlusion.
- Confirmed symptomatic intracranial occlusion at one or more of the following locations: Intracranial carotid I/T/L, M1 or M2 segment MCA.

Main Exclusion Criteria

- Treated with a tissue plasminogen activator within 24 hours before randomization.
- Determination by the treating physician, based on current treatment guidelines and medical evidence, that treatment with a plasminogen activator is indicated.
- Large core of established infarction defined as ASPECTS 0-4.
- Absent or poor collateral circulation on qualifying imaging (e.g., Collateral score of 0 or 1).

Test product, dose and mode of administration, batch number:	Nerinetide CMPLI028-19 2.6 mg/kg of active drug (up to a maximum of 270 mg), as a single 10 ± 1-minute intravenous (IV) infusion.
Reference therapy, dose and mode of administration, batch number:	Placebo CMPLI028-19 Phosphate buffered saline of equivalent volume given as single 10 ± 1-minute IV infusion.
Duration of treatment:	Participants received a single 10-minute infusion of study drug on Day 1. Each participant was followed in the main trial for 90 days. Some participants were followed for 1 year for the One-year sub-trial.

Name of Sponsor/Company: NoNO Inc.	(For National Authority Use only)
Name of Finished Product: Nerinetide (NA-1, 2.6 mg/kg)	
Name of Active Ingredient: NA-1	

Criteria for evaluation:

Efficacy:

Primary:

The primary endpoint used in this trial was global disability, as measured by the modified Ranking Scale (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. The mRS scores range from 0 to 6, with 0 indicating no residual symptoms; 5 indicating bed-bound, requiring constant care; and 6 indicating death.

***Secondary*:**

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

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

The National Institutes of Health Stroke Scale (NIHSS) is a standardized neurological examination score that is a valid and reliable measure of disability and recovery after acute stroke. 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.

***Tertiary*:**

Volume of Stroke the total lesion volume of stroke as measured by MRI or CT brain images (MRI preferred) in nerinetide versus placebo groups was calculated from the Day 2/3 imaging.

The Barthel Index (BI) is an index of functional independence that is a valid measure of activities of daily living when employed in stroke trials. Modified BI scores range from 0 to 100, with higher scores indicating greater independence in activities of daily living and mobility.

The European Quality of Life (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 5 dimensions: mobility, self-care, usual activities, pain/discomfort, and anxiety/depression. Each dimension has 5 response categories corresponding to: no problems, slight, moderate, severe, and extreme problems. The version of the instrument selected for the trial was interviewer administered either in-person, by telemedicine, or by telephone. The respondents also rated their overall health on the day of the interview on a 0–100 visual analog scale (EQ-VAS). The assessment was only conducted if the available in the local country language.

Safety:

Frequencies of SAEs and 90-day mortality.

Name of Sponsor/Company: NoNO Inc.	(For National Authority Use only)
Name of Finished Product: Nerinetide (NA-1, 2.6 mg/kg)	
Name of Active Ingredient: NA-1	

Statistical methods:

Primary Efficacy:

The primary analysis for the primary estimand was conducted in the Intent-to-Treat (ITT) population according to the randomized treatment. Missing data were imputed following the single imputation approach. The primary hypothesis tested was that administration of nerinetide would 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 was 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 was the method of Ge et al, 2011. The difference in responder rate between 2 treatment groups and the associated standard error and 95% confidence interval (CI) was reported. P-value for the primary endpoint was calculated based on the estimates reported by the Ge et al, 2011 method.

The pivotal primary analysis model was 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 (i.e., age, baseline National Institutes of Health Stroke Score (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 was not significant at the level of 0.05, it was removed from the model. The odds ratios along with the 95% CI was reported in addition to the primary Ge et al, 2011 method results. If the interaction term was significant, results were also reported by randomization stratum separately, using the Ge et al, 2011 method.

Secondary Efficacy:

The analysis for the secondary estimands with binary endpoints was 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. Summary statistics for each tertiary efficacy endpoint were tabulated by treatment group.

***Tertiary Efficacy*:**

The tertiary analyses were considered exploratory, and therefore were not participant 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 were reported as described for each outcome including reporting by randomization strata if the interaction term in the adjusted logistic regression model used for the primary estimand was significant at the 0.05 level. The odds ratio, 95% CI, and p-value from the logistic regression model were reported.

Safety:

Name of Sponsor/Company: NoNO Inc.	(For National Authority Use only)
Name of Finished Product: Nerinetide (NA-1, 2.6 mg/kg)	
Name of Active Ingredient: NA-1	

All safety analyses was performed on the Safety Population. The main analyses were frequency of SAEs and 90-day mortality. Additional analyses included analyses of vital signs and clinical laboratory values.

Pharmacokinetic:

Descriptive statistics were calculated for plasma concentrations and for all PK parameters (nerinetide). Samples with no detectable nerinetide were excluded from analysis (placebo). Individual and mean plasma concentration versus time curves were plotted on linear and semi-logarithmic scales.

SUMMARY DISPOSITION, DEMOGRAPHIC, BASELINE CHARACTERISTICS, AND COMPLIANCE

Between 06 December 2020 and 31 January 2023, 850 participants were enrolled and randomized to receive nerinetide or placebo. In total 844 participants received study drug: 451 (99%) participants in the nerinetide group and 393 (99%) participants in the placebo group. In total, 77 principal investigators at 77 sites in 9 countries (Canada, USA, Germany, Italy, Netherlands, Norway, Switzerland, Australia and Singapore) enrolled subjects in this global multicenter study.

Overall, 98% of participants completed the trial. The main reason for early termination was 'Withdrawal by participant' by 12 participants and 6 participants were 'Lost to follow up'. Slightly fewer participants in nerinetide group (441 participants, 97%) completed the trial to Day 90 compared to the placebo group (391 participants, 99%). Day 90 primary outcomes were missing for 18 (2%) participants; 12 (1%) withdrew consent and 6 (0.7%) were lost to follow-up. The rates were slightly higher in the nerinetide group (13 participants, 3%) compared to the placebo group (5 participants, 1%).

Demographics and baseline characteristics were similar in the nerinetide and placebo groups. The mean age was 73.2 ±12.96, years, 50% of participants were female, 98% were non-Hispanic, and 81% were white, 7% Asian and 4% black. The median baseline NIHSS score was 16 and 67% had a baseline ASPECTS score of 8-10. The occlusion location was the MCA-M1 in 68% of participants. Stroke at awakening occurred in 17% of participants and 58% of all enrolled participants had their stroke onset witnessed.

The time from stroke onset to randomization ≤4.5 hours occurred in 52% of participants, with slightly more participants in the nerinetide group (56%) compared to placebo (48%). The mean time from qualifying imaging was similar in both groups 0.78 hours (47 minutes).

A total of 58 important protocol deviations occurred which resulted in the participant being removed from the per protocol analysis. Similar percentages of participants had at least 1 protocol deviation in the nerinetide (30 participants, 7%) and placebo group (28 participants, 7%). Important protocol deviations that removed participants from the PP analysis included: inclusion criteria not met and study drug dose volume not compliant.

EFFICACY RESULTS

In the population studied, nerinetide was not effective in reducing disability from ischaemic stroke due to large vessel occlusion at 90 days in participants selected for EVT over a 12 hour enrollment window without prior thrombolysis.

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Name of Finished Product: Nerinetide (NA-1, 2.6 mg/kg)	
Name of Active Ingredient: NA-1	

Item	Nerinetide (N=454)	Placebo (N=396)	Total (N=850)
mRS 0-2 (n [%])	206 (45)	181 (46)	387 (46)
mRS >2 (n [%])	248 (55)	215 (54)	463 (54)
Odds Ratio	0.959		
95% CI	(0.715,1.288)		
p-value	0.782		

Secondary outcomes were similarly neutral in the overall population. These results failed to confirm those obtained in participants in the no-alteplase stratum of the ESCAPE-NA1 trial. In that study, treatment with nerinetide was associated with an absolute reduction in functional disability (mRS>2) of 9.6%, a 7.5% absolute reduction in mortality, and a 22% relative reduction in infarction volumes.

In the post database-lock, ad hoc analysis of participants enrolled in the first three hours after stroke onset there was a numeric benefit for participants who received nerinetide. Specifically, treatment with nerinetide resulted in better functional outcomes as measured with the modified Rankin Scale when administered within 3 hours of stroke onset.

SAFETY RESULTS:

The safety population consisted of any enrolled participants who received any amount of study drug. In this trial, there were no notable differences in rates of TEAEs in participants treated with nerinetide compared with placebo in the safety population as a whole, suggesting an acceptable safety profile. No other differences in other important safety outcomes were observed, overall and stratified by alteplase use.

The results of the safety analysis indicate that nerinetide has a safety profile comparable to placebo and was well tolerated when given as a single IV dose of 2.6 mg/kg to participants with acute ischemic stroke. Summary of specific observations relating to an acceptable safety profile of nerinetide is presented below:

Deaths: The frequency of TEAEs resulting in death was the same in the nerinetide and placebo groups (17% in both groups).

TEAEs: the overall frequency of TEAEs was similar in both treatment groups (87% in the nerinetide group versus 86% in the placebo group). On the basis of MedDRA preferred terms the most frequent TEAEs (≥ 10% of subjects in nerinetide group) were haemorrhagic transformation stroke (13% in nerinetide vs 11% in placebo), urinary tract infection (12% in nerinetide vs 12% in placebo), hypotension (12% in nerinetide vs 9% in placebo), and hypokalaemia (10% in nerinetide vs 9% in placebo).

AESIs: the overall frequency of AESIs was similar between the nerinetide and placebo groups (8% and 7%, respectively), with the most frequently reported AESI being hypotension (33 participants, 7% in nerinetide group vs 25 participants, 6% in placebo group).

TEAS by maximum severity: Overall, the majority of TEAEs were assessed as either mild or moderate in severity. The frequency of severe TEAEs was similar between the nerinetide and placebo groups (33% and 32% respectively).

Name of Sponsor/Company: NoNO Inc.	(For National Authority Use only)
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Name of Active Ingredient: NA-1	

Laboratory safety parameters: Baseline (pre-dose) and Day 2/3 clinical laboratory assessments were evaluated, this includes: CBC (hemoglobin, platelets and hematocrit), chemistry (serum glucose, serum creatinine) and electrolytes (sodium, potassium, chloride). At Day 2/3 there were no differences in the median values between nerinetide and placebo for any of the parameters. There was a similar change at Day2/3 from baseline in both groups for hemoglobin (increase of 13.21 g/L) and platelets (decrease of -20.0 10^9/L).

CONCLUSION:

In conclusion, treatment with single 2.6 mg/kg IV dose of nerinetide did not improve the proportion of good clinical outcomes among endovascular thrombectomy patients not previously treated with thrombolytics over a 12 hour enrollment window. Further studies in patients selected for EVT should consider designs in which neuroprotection can be administered early enough to show a detectable difference in outcomes by slowing stroke progression.