Efficacy and safety of intravenous nerinetide initiated by paramedics in the field for acute cerebral ischaemia within 3 h of symptom onset (FRONTIER): a phase 2, multicentre, randomised, double-blind, placebo-controlled study

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Summary

Background Nerinetide is a neuroprotectant effective in preclinical models of acute ischaemic stroke when administered within 3 h of onset. However, the clinical evaluation of neuroprotectants in this short timeframe is challenging. We sought to establish the feasibility, safety, and effectiveness of nerinetide when given before hospital arrival within 3 h of symptom onset of suspected stroke.

Methods In this multicentre, randomised, double-blind, placebo-controlled study, paramedics enrolled participants aged 40–95 years within 3 h of suspected severe stroke onset, who were previously independent, and were being taken to one of seven stroke centres in Ontario or British Columbia, Canada. The primary hypothesis was that the administration of nerinetide would result in a higher rate of good functional outcomes. Participants were randomly assigned 1:1 to intravenous nerinetide (2·6 mg/kg) or placebo, each in visually identical vials. Paramedics, hospital care providers, and outcome evaluators were masked to treatment assignment. The primary outcome was good functional outcome on a sliding dichotomy of the modified Rankin Scale at 90 days. Participants were assessed on day 4, 30, and 90 by the stroke center research team, in person or over the telephone. Outcomes, adjusted for age and stroke severity, were evaluated in the modified intention-to-treat (mITT) population, and in the target population of those with acute ischaemic stroke. The safety population included all participants who received the study drug. This study is registered with ClinicalTrials.gov (NCT02315443), and trial enrolment has concluded.

Findings Between March 26, 2015, and March 27, 2023, 532 participants received nerinetide (n=265) or placebo (n=267). The mITT population of suspected stroke (n=507; 254 nerinetide and 253 placebo) included 321 (63%) with acute ischaemic stroke, 93 (18%) with intracranial haemorrhage, 44 (9%) with transient ischaemic attack, and 49 (10%) with stroke-mimicking conditions. Treatment began a median of 64 min (IQR 47–100) from symptom onset. Participants randomly assigned to nerinetide had more severe strokes compared with those receiving placebo (median National Institutes of Health Stroke Scale (NIHSS) 12, IQR 5–19 *vs* 10, 4–18 in mITT, and 14, 7–19 *vs* 10, 4–18 in the acute ischaemic stroke subgroup). Overall, 145 (57%) of 254 participants in the nerinetide group and 147 (58%) of 253 in the placebo group had the primary outcome of a favourable functional outcome using the prespecified sliding dichotomy at 90 days (adjusted odds ratio 1·05, 95% CI 0·73–1·51; adjusted risk ratio 1·04, 95% CI 0·85–1·25). In the 302 patients with ischaemic stroke, the favourable functional outcome adjusted for arrival NIHSS and age favoured nerinetide (odds ratio 1·53, 0·93–2·52 and risk ratio 1·21, 0·97–1·52). In those given reperfusion therapies (thrombolysis or endovascular thrombectomy, or both) nerinetide was associated with improved favourable functional outcomes (adjusted odds ratio 1·84, 1·03–3·28; adjusted risk ratio 1·29, 1·01–1·65). There was no apparent benefit in haemorrhagic stroke or acute ischaemic stroke without reperfusion. There were no safety concerns.

Interpretation Prehospital nerinetide did not improve neurological functional outcomes in all patients with suspected ischaemic stroke in the mITT population. Nerinetide might benefit patients with acute ischaemic stroke who are selected for reperfusion therapies within 3 h of symptom onset. This finding should be confirmed in a future trial.

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Research in context

Evidence before this study

We searched PubMed for articles published in English from database inception up to July 23, 2024, investigating the neuroprotectant nerinetide for the treatment of clinical and experimental stroke, using the following search terms: "nerinetide", "NA-1", "Tat-NR2B9c", "PSD-95 inhibitor", AND "stroke". We also searched US Food and Drug Administration and European Medicines Agency drug approval databases for neuroprotectant drugs for the treatment of stroke. At the time of planning, all previous trials of potential neuroprotective agents delivered the treatment more than 4 h after symptom onset, except for the FAST-MAG trial of magnesium administered by paramedics and continued for 24 h in hospital. No studies showed a benefit leading to broad clinical use. Four recent trials of neuroprotective strategies in the prehospital setting have all been neutral (MR ASAP, RIGHT-2, INTERACT-4, and RESIST).

Added value of this study

The FRONTIER trial design was based on a large series of animal studies suggesting a significant benefit for neuroprotection provided by nerinetide within a 3 h treatment window. The ESCAPE-NA1 and ESCAPE-NEXT studies were completed during the FRONTIER trial, but could not examine whether nerinetide could be useful in conjunction with thrombolytic agents because ESCAPE-NEXT focused on patients not given thrombolytics, and ESCAPE-NA1 participants who received thrombolysis received it before nerinetide was given, probably resulting in its cleavage and inactivation by plasmin. By contrast, participants in FRONTIER received nerinetide before arriving to hospital and receiving thrombolysis, enabling the interrogation of the synergism between neuroprotection and reperfusion with a thrombolytic. The FRONTIER trial provided nerinetide in a randomised and masked method from paramedics to patients with suspected stroke soon after the onset of symptoms, making it the first such prehospital trial of an investigational new drug in acute ischaemic stroke. It showed the feasibility of enrolling and treating participants with an investigational new drug by primary care paramedics in the ambulance, outside of the hospital setting. This approach had the advantage of a short interval from symptom onset to enrolment, but the disadvantage of enrolling a heterogeneous patient population, with many participants having diagnoses other than that of acute ischaemic stroke, which reduced the statistical power of the study as a whole. However, enrolling such patients showed the acceptable safety of nerinetide not only in patients with ischaemic stroke but also in those with haemorrhagic stroke, transient ischaemic attack, and in patients with various stroke-mimicking conditions, which is important when the enrolment strategy is suspected (but not yet proven) stroke. Exploratory analyses of several clinically meaningful endpoints consistently resulted in better outcomes in the population of patients with ischaemic stroke receiving reperfusion therapy, and with the greatest effect seen when reperfusion included a thrombolytic agent.

Implications of all the available evidence

Although nerinetide was not beneficial to all patients with a suspected stroke, it appeared to provide a clinical benefit to patients with the target disease of acute ischaemic stroke, as an adjunct to reperfusion therapy. The greatest benefit appeared to be in those receiving thrombolytic therapy. Since thrombolytics do not instantly open the occluded artery, nerinetide might prevent ongoing cell death during that period of ongoing ischaemia before clot dissolution. The hypothesis that neuroprotectants are especially useful when instilled early and during a period of ongoing ischaemia, before artery recanalisation, should be tested in a future trial. FRONTIER potentially supports a strategy that would be effective in such patients that are not in proximity to a stroke centre capable of endovascular intervention.

Introduction

Acute ischaemic stroke is a time-sensitive emergency most commonly due to the blockage of an artery to the brain by a thrombus. Blood flow restoration, using intravenous thrombolysis or endovascular thrombectomy, or both, improves stroke outcome if reperfusion is achieved before ischaemic damage is complete. Even among patients who seek assistance early, reperfusion occurs hours after stroke onset. Too often, transport requirements, in-hospital processes, or technical complexities delay endovascular thrombectomy, which even in the best centres is unsuccessful in approximately 10% of individuals. Similarly, thrombolysis achieves recanalisation within 2 h of drug administration in only 10–20% of individuals,[1–3] and this number only reaches up to 70% after 24 h.[4] During the time from symptom onset to reperfusion, stroke continues to progress,[5] leading to greater clinical disability.

Neuroprotection, a treatment that slows stroke progression by enhancing the brain's resilience to ischaemia, could reduce the adverse effect of the ongoing ischaemia until reperfusion occurs.[6] Nerinetide is a synthetic peptide neuroprotectant designed for this purpose. It binds to postsynaptic density 95 protein in central neuronal synapses, perturbing the linkage between N-methyl-D-aspartate glutamate receptors and downstream signalling proteins that mediate excitotoxicity, including neuronal nitric oxide synthase.[7] Nerinetide limits ischaemic brain damage in experimental animals including mice,[8] rats,[9] and primates,[10] and is most effective when reperfusion occurs within 3 h of ischaemia onset.

The FRONTIER trial sought to conform to the preclinical science by enrolling participants within 3 h of symptom onset, and anticipated that many would receive reperfusion therapy. The earliest practical time for

initiating neuroprotection is at the time of paramedic assessment in the field. However, the trade-off for the prehospital enrolment of individuals with suspected stroke was the inclusion of participants who would not require neuroprotection (transient ischaemic attack or stroke-mimicking conditions) and some who would not probably benefit from anti-ischaemic agents (haemorrhagic stroke). Although the inclusion of potential non-responders limited the statistical power of the trial to detect benefit in the overall cohort, it provided an opportunity to explore safety and efficacy and various endpoints in important subpopulations, a key goal of a phase 2 trial. Prehospital enrolment also ensured that the research protocol did not compete with in-hospital, time-sensitive, standard-of-care treatments including thrombolysis and endovascular thrombectomy.

The trial was designed to establish whether early treatment with nerinetide delivered in the prehospital setting, in addition to usual care with thrombolysis or endovascular thrombectomy, or both, as indicated, would improve outcomes for all patients presenting with signs and symptoms of acute stroke and in those with confirmation of ischaemic stroke.

Methods

Study design

FRONTIER was a multicentre, randomised, double-blind, placebo-controlled, single-dose, phase 2 clinical trial to assess the efficacy and safety of intravenous nerinetide in patients with suspected stroke within 3 h of symptom onset. The study was conducted in Canada, in sites with experienced ambulance services who delivered patients with stroke to established stroke centres. It was approved by ethics committees at all sites to use a waiver of consent, a process used in other Canadian emergency resuscitation trials.[11] Consent was required from the participant or a legally authorised representative after arrival in hospital for follow-up procedures and use of data for 90 days. For individuals that declined participation or withdrew consent, the use of any participant data after withdrawal was precluded. The consent process is described in more detail in the appendix (pp 5, 70). The University of British Columbia Harmonized Research Ethics Board was the main board that approved this study (certificate number H14–01296). Multiple local Research Ethics Boards also provided approval for their involvement in the study. Nerinetide or placebo was administered by paramedics before hospital arrival. Throughout the enrolment period, there were no changes in the protocol other than accommodations for remote assessments of endpoints during the COVID-19 pandemic. The trial is registered with ClinicalTrials.gov (NCT02315443).

Participants

Eligible patients were adults aged 40–95 years with a suspected stroke who could receive the study drug within 3 h of symptom onset, were being taken to transfer to a study stroke centre, were independently ambulatory before symptom onset, and had a stroke severity score on the Los Angeles Motor Scale (LAMS; range 0 [no symptoms] to 5 [most severe])[12] of 2–5 for 15 min or more, as well as a score of 2–5 at the start of study drug infusion. The exclusion criteria were: a Canadian Triage Scale of level 1 (requiring urgent resuscitation), seizure at symptom onset or witnessed by the paramedic, Glasgow Coma Score of less than 10, oxygen saturation of less than 90% on room air, respiratory rate of less than 12 or more than 24 breaths per minute, weight of less than 45 kg or more than 120 kg, blood glucose of less than 3 mmol/L, major head trauma or stroke within the past 3 months, known or suspected pregnancy, being in long-term care, known advanced care directive to not resuscitate, known previous disease that precluded obtaining a final positive neurological outcome, or study drug temperature warming more than 8°C for more than 48 h.

The study was conducted by three emergency medical services agencies in Canada (Peel Paramedic Services, Toronto Paramedic Services, and British Columbia Emergency Health Services) and seven stroke centres across Ontario (St Michael's Hospital, Sunnybrook Health Science Centre, Toronto Western Hospital, and Trillium Health Partners) and British Columbia (Kelowna General Hospital, Royal Columbian Hospital, and Vancouver General Hospital).

Paramedics identified potentially eligible individuals with suspected stroke using their regional stroke recognition tools (Los Angeles Prehospital Stroke Screen[13] in Ontario and The Cincinnati Prehospital Stroke Scale[14] in British Columbia) per clinical protocols. They then reviewed the criteria for enrolment. Potentially eligible individuals at all sites were subsequently evaluated using the LAMS for stroke severity. If paramedics deemed that the patient might be a good candidate, they contacted the on-call physician for the trial (stroke neurologist or emergency medical services physician) to confirm that the individual met all inclusion criteria and did not meet any exclusion criteria, to authorise enrolment and study drug dose. The enrolment process did not affect any aspect of standard patient care, including destination.

See Online for appendix

Randomisation and masking

Vial allocation and therefore random assignment was done by a prespecified permuted block design, stratified by emergency medical services regional coordinating centre, with a block size of 2. Nerinetide and placebo vials were visually identical. A drug vial allocation sequence, generated using a random number generator by an independent third party statistician, was used by the manufacturer to consecutively number the visually identical drug or placebo vials, which were packaged into boxes containing 42 consecutive vials allocating nerinetide or placebo in a 1:1 ratio. All other trial staff,

including the paramedics, health-care team, all investigators, monitors, and the sponsor had no access to this sequence before database lock and were thus masked to the intervention. Each emergency medical services centre received single boxes of 42 vials. A single drug vial was allocated to each participating ambulance at the start of the trial and, after use, restocked with the next consecutive vial from the box. Thus, paramedics enrolled participants into the sole intervention (nerinetide or placebo) available in the ambulance at the time. This method was designed to minimise errors in treatment allocation but precluded any methods for balancing the randomisation based on demographics, actual diagnosis, or stroke severity.

Interventions

Patients received either saline placebo or nerinetide in a single dose of 2·6 mg/kg, up to a maximum dose of 270 mg, using estimated or known bodyweight. Nerinetide or placebo was delivered through a dedicated intravenous line, over 10 (±1) min by infusion pump. The time of random assignment was the moment that the participant received any study drug. Upon emergency department arrival, participants were evaluated and received usual care, as indicated by their diagnosis, including thrombolysis or endovascular thrombectomy, or both, as necessary. Thus, none of the participants received thrombolysis or endovascular thrombectomy before receiving the study drug.

Outcomes

The primary outcome was a responder analysis using a sliding dichotomy defined on the modified Rankin Scale (mRS; range 0 [no symptoms] to 6 [death]).[15–17] A responder was defined as an individual having an mRS score of 0–2, except for participants younger than 80 years with a LAMS of 2–3, in whom it was an mRS score of 0–1. Secondary outcomes were: a shift across the mRS scale to a lower score, reflecting reduced functional dependence, analysed across the whole distribution of scores on the mRS, with scores of 5 and 6 collapsed into a single category;[18] mortality; day 90 National Institutes of Health Stroke Scale (NIHSS) score; and worsening of stroke. Worsening was defined as progression or haemorrhagic transformation of the index stroke that: (1) was deemed life-threatening or (2) resulted in increased disability as gauged by a 4 or more point increase from the lowest NIHSS score (range 0–42, with higher scores indicating greater stroke severity)[19] during hospitalisation or (3) resulted in death, or a combination of these three. Additional outcomes of interest were: excellent functional outcome, defined by an mRS score of 0–1; functional independence in activities of daily living, defined by a Barthel index score of 95 or higher; health-related quality of life, as measured by the EuroQol validated assessment of quality of life over 5 dimensions each with 5 levels (EQ-5D-5L) and EuroQual validated visual analogue score from 1 to 100 as an overall assessment of quality of life on the day of the assessment (EQ-VAS); and assessments of stroke volumes on 24 h imaging (MRI or CT). Worsening of stroke and mortality were assessed according to the prescribed evaluation timetable. Outcomes were assessed by personnel certified in the scoring methods 90 days after random assignment in person or, if an in-person visit was not possible, by telephone. Safety outcomes were all serious adverse events, worsening of stroke, and mortality. A schedule of assessments is provided in the appendix (p 25).

Statistical analysis

Sample size projections were based on powering the study at 80% for a dichotomous outcome (responder *vs* non-responder). Based on the FAST-MAG trial[20] it was predicted that approximately 72% of randomly assigned participants would have acute ischaemic stroke, 24% would have intracerebral haemorrhage as their stroke subtype, and 4% would have stroke-mimicking conditions. Assuming a 26% overall responder rate (based on day 90 outcomes anticipated for patients expected to be enrolled with the various diagnoses expected in the trial, including ischaemic and haemorrhagic strokes, weighted in accordance with the anticipated proportions of such diagnoses in the trial) for the placebo group using the sliding dichotomy definition of responder, there would be an estimated 80% power to detect a 12% absolute effect difference between response rate (proportion of responders) with nerinetide and placebo, at α level 0·05, two-sided with a planned sample size of 506 evaluable participants, randomly assigned 1:1, 253 per group. The 12% absolute response rate difference was judged to be a clinically important difference to justify the prehospital administration of nerinetide. The sample size was inflated 10% to account for loss-to-follow-up and dropouts (up to n=558; 279 per group). There was no planned interim analysis for efficacy.

Statistical analyses were conducted concurrently by a third party external statistical consulting group (funded by NoNO [Toronto, ON, Canada]) and by the academic investigators. The primary analysis was done on the modified intention-to-treat (mITT) population, defined as any participant with at least one mRS assessment done after a dose of the study drug, and was a multivariable logistic regression adjusted for treatment and the variables of emergency medical services coordinating centre, age, and baseline LAMS score. Treatment effects were reported as adjusted odds ratios and adjusted risk ratios with their 95% CIs. A hierarchical approach was used to control for multiplicity, beginning with the primary outcome (responders) and proceeding to secondary outcomes in the following order: shift analysis of 90-day mRS using a proportional odds model across the mRS scale, mortality, and worsening of stroke. Assessment of day 90 NIHSS outcomes, prespecified in the statistical

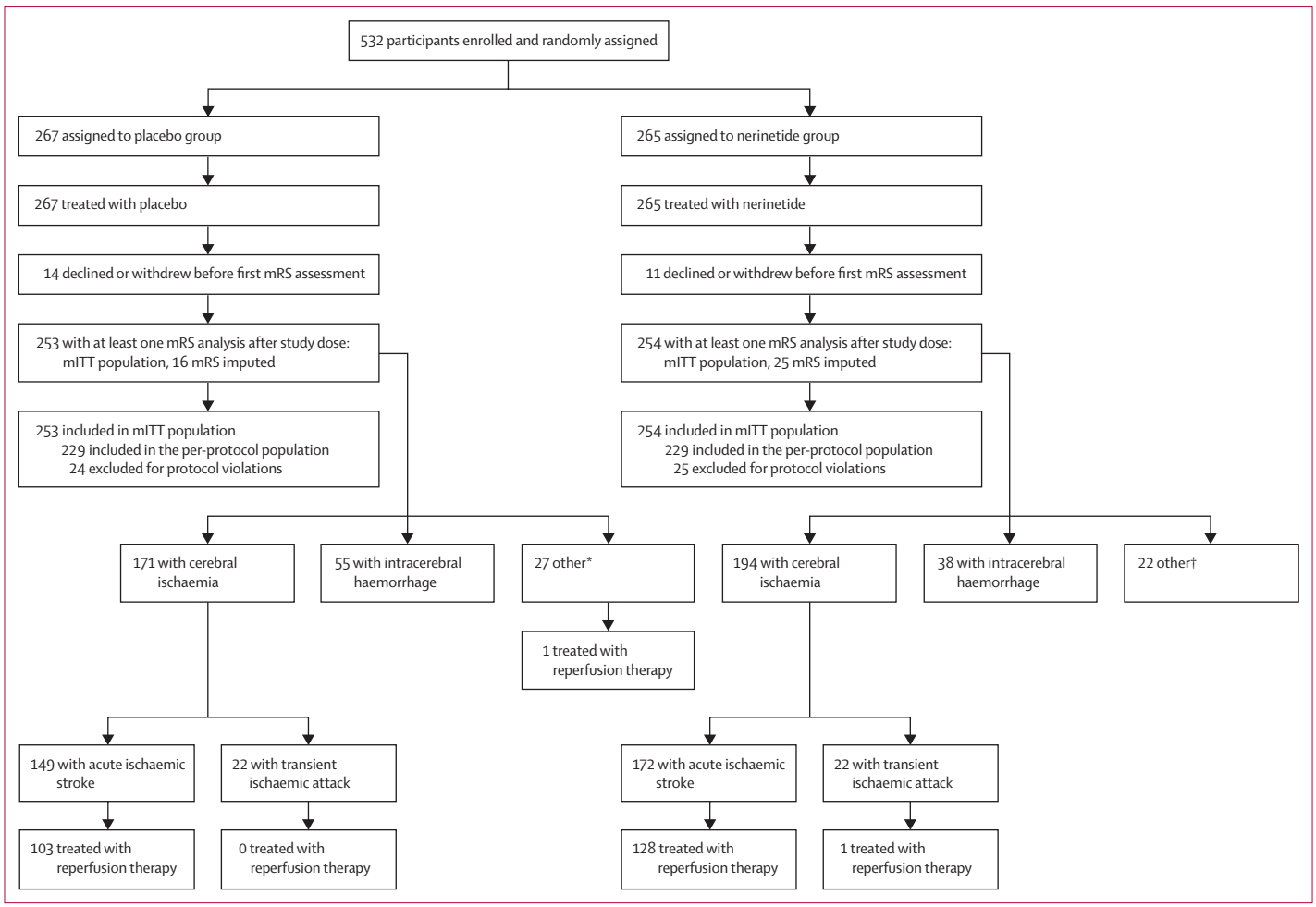

Figure 1: **Consort diagram**
mITT=modified intention-to-treat. mRS=modified Rankin Scale. *24 mimics and three undetermined. †20 mimics and two undetermined.

analysis plan, was not conducted due to inability to conduct in-person assessments during the COVID-19 pandemic. Deceased participants were considered to have completed the trial and were included in the mITT population with an mRS score of 6, a Barthel index score of 0, and an NIHSS score of 42. Missing day 90 mRS outcomes were imputed to the day 30 mRS if that was available, otherwise an mRS of 6 was used if both day 30 and day 90 scores were missing, or an mRS of 5 was used if the scores were missing but the participant was documented to be alive. For missing day 90 mortality analysis, participants were imputed as alive if known to be alive at day 30, but otherwise imputed as dead. All outcomes at and after the demonstration of no difference in the primary outcome with a two-sided p value of more than 0·05 were considered exploratory and were not adjusted for multiplicity or imputed.

Exploratory analyses were adjusted for age and NIHSS upon emergency department arrival, two crucially important prognostic covariates for stroke.[21,22] These differed from the statistical analysis plan, in which the outcomes were adjusted for stroke severity using the baseline LAMS score, since the NIHSS was out of scope in paramedic practice. The rationale for replacing the LAMS with the emergency department arrival NIHSS was that, unlike in the FAST-MAG study,[23] the LAMS did not correlate with outcome and was of limited use for adjusting for differences in stroke severity between the nerinetide and placebo groups (appendix pp 18–19). The LAMS was used for the prespecified primary analysis, compliant with the statistical analysis plan; only exploratory analyses used the NIHSS instead. Exploratory analyses were conducted on the following populations identified in the statistical analysis plan: the mITT population; per-protocol participants in the mITT population with no major protocol deviations; and the following subgroups: those with a final diagnosis of acute ischaemic stroke; those who received any reperfusion therapy (thrombolysis or endovascular thrombectomy, or both); those who received thrombolysis; those who received endovascular

	Nerinetide (n=254)	Placebo (n=253)	Total (n=507)
Age, years	74 (65–81)	75 (64–83)	75 (64–82)
Sex			
Men	147 (58%)	141 (56%)	288 (57%)
Women	107 (42%)	112 (44%)	219 (43%)
Race			
White	159 (63%)	158 (62%)	317 (63%)
Black or African American	4 (2%)	7 (3%)	11 (2%)
Asian	82 (32%)	81 (32%)	163 (32%)
Other	9 (4%)	7 (3%)	16 (3%)
Medical history			
Cardiovascular	158 (62%)	149 (59%)	307 (61%)
Congestive heart failure or pulmonary oedema	22 (9%)	28 (11%)	50 (10%)
Peripheral vascular disease	16 (6%)	29 (11%)	45 (9%)
Vascular risk factors			
Hypertension	186 (73%)	181 (72%)	367 (72%)
Diabetes	63 (25%)	56 (22%)	119 (23%)
Hyperlipidaemia	124 (49%)	123 (49%)	247 (49%)
Atrial fibrillation	73 (29%)	64 (25%)	137 (27%)
Tobacco, current or within past year	45 (18%)	44 (17%)	89 (18%)
Coronary artery bypass graft or coronary angioplasty	27 (11%)	24 (9%)	51 (10%)
Previous cerebral infarcts	74 (29%)	46 (18%)	120 (24%)
Previous transient ischaemic attack	39 (15%)	28 (11%)	67 (13%)
Previous intracerebral haemorrhages	9 (4%)	8 (3%)	17 (3%)
Previous stroke, infarct *vs* haemorrhage unknown	23 (9%)	18 (7%)	41 (8%)
Renal disease	37 (15%)	34 (13%)	71 (14%)
Dialysis	1 (<1%)	0	1 (<1%)
Existing hemiplegia or paraplegia	8 (3%)	8 (3%)	16 (3%)
Clinical characteristics			
Type of stroke			
Intracerebral haemorrhage	38 (15%)	55 (22%)	93 (18%)
Ischaemic	172 (68%)	149 (59%)	321 (63%)
Transient ischaemic attack	22 (9%)	22 (9%)	44 (9%)
Stroke-mimicking condition or undetermined	22 (9%)	27 (11%)	49 (10%)
Stroke severity			
Baseline LAMS	4 (3–5)	4 (3–5)	4 (3–5)
Baseline NIHSS	12 (5–19)	10 (4–18)	11 (4–18)
Participants without acute ischaemic stroke, baseline NIHSS*	8 (2–19)	9 (2–18)	9 (2–19)
Reperfusion received			
Intravenous thrombolysis	116 (46%)	96 (38%)	212 (42%)
Endovascular thrombectomy	53 (21%)	45 (18%)	98 (19%)
Intravenous thrombolysis or endovascular thrombectomy, or both; any reperfusion	129 (51%)	104 (41%)	233 (46%)
Workflows			
Last seen normal to dosing, min	60 (45–95)	68 (49–105)	64 (47–100)
Dosing to intravenous thrombolysis, min†	54 (41–69)	56 (43–66)	55 (42–67)
Dosing to endovascular thrombectomy, min‡	104 (77–129)	95 (77–131)	102 (77–130)
Dosing to emergency department arrival, min	12 (5–17)	10 (5–16)	11 (5–16)

Data are median (IQR) or n (%). LAMS=Los Angeles Motor Scale. NIHSS=National Institutes of Health Stroke Scale score. *68 received nerinetide and 92 received placebo. †116 received nerinetide and 96 received placebo. ‡53 received nerinetide and 45 received placebo.

Table 1: **Demographics and clinical characteristics**

thrombectomy; those with acute ischaemic stroke without reperfusion therapy; and those with a final diagnosis of intracerebral haemorrhage.

For analyses of health-related quality of life (EQ-5D-5L), responses on each of the five domains were converted to Canadian utilities[24] (ie, values that measure how much a person values their health status) to generate EQ-5D-5L index scores. Deaths were imputed to have EQ-5D-5L index and VAS scores of 0. Index scores and VAS scores in treatment and control groups were compared using quantile regressions for multivariable analyses, adjusted for age and NIHSS.

The safety population included all participants who received any amount of study drug. This trial was monitored by an independent data monitoring committee, which conducted safety analyses at 25 participants, 50 participants, and 300 participants. Analyses were done using SAS software (version 9.4) or STATA (version 16.0). Details are provided in the statistical analysis plan provided in the appendix (appendix pp 145–150).

Role of the funding source

The regulatory sponsor and co-funder of the study (NoNO) participated in the study design, analysis, interpretation, and writing of the report. All other funders of the study had no role in data collection, data analysis, data interpretation, or writing of the report.

Results

Between March 26, 2015, and March 27, 2023, 532 participants being taken to one of seven stroke centres in Canada were randomly assigned to receive nerinetide (n=265) or placebo (n=267). 25 participants (5%) did not have at least one mRS score after a study dose because they declined participation upon hospital arrival or withdrew consent (11 nerinetide and 14 placebo; appendix p 7) and were not included in the mITT analysis. Withdrawals occurred most frequently in participants with stroke-mimicking conditions (seven [14%] of 51 participants) compared with ischaemic (15 [4%] of 336) or haemorrhagic stroke (two [2%] of 95). The prespecified mITT population comprised 507 participants (254 nerinetide and 253 placebo); 321 (63%) had acute ischaemic stroke, 44 (9%) had transient ischaemic attack, 93 (18%) had haemorrhagic stroke (intracerebral haemorrhage), and 49 (10%) had stroke-mimicking conditions or other diagnoses (figure 1). The trial ceased enrolment at 532 due to slow enrolment during the COVID-19 pandemic.

Baseline demographics and past medical history were similar between the two groups (table 1; appendix pp 11–16). There were two notable imbalances between the nerinetide and placebo groups. First, there was an imbalance in baseline stroke severity, as gauged by NIHSS score on emergency department arrival, in that strokes were more severe in the nerinetide group as compared with placebo (table 1, 2). This difference was driven largely by participants with acute ischaemic

stroke, whose median NIHSS was 14 (IQR 7–19) in the nerinetide group compared with 10 (4–18) in the placebo group (Mann–Whitney *U*, p=0·024; table 2). Second, more participants with a diagnosis of acute ischaemic stroke received nerinetide versus placebo (172 *vs* 149, respectively; Fisher's exact, p=0·043).

Two participants in the nerinetide and four in the placebo group received an incomplete dose of the study drug. 454 (90%) of 507 participants received their infusion over 10 min (SD 1). There were no crossovers. A summary of exposure and compliance is provided in the appendix (p 17).

The mean time intervals from symptom onset to initiation of study drug infusion were similar between groups: 62·5 min (SD 31) for nerinetide and 65·5 min (SD 31) for placebo (p=0·290). The interval from dosing initiation to emergency department arrival was 11·0 min (9·6). For participants who received thrombolysis, dosing initiation to thrombolysis start averaged 57·6 min (22·6) and for those who received endovascular thrombectomy, study drug infusion to endovascular thrombectomy start was 138·8 min (246). There were no differences between the nerinetide and placebo groups in thrombolysis or endovascular thrombectomy process times. A summary of process times is provided in table 1 for the mITT population and by final diagnosis subpopulations in the appendix (pp 11–16).

The day 90 mRS score was imputed for 41 participants (8%) in the mITT population as per the statistical analysis plan (appendix pp 8–9), with no differences in the rate of imputation between nerinetide and placebo (Fisher's exact, p=0·192; appendix p 8). Data were imputed least frequently in the acute ischaemic stroke population (14 [4%] of 321 participants) and most frequently in stroke-mimicking conditions (14 [32%] of 44; appendix p 9). Protocol deviations occurred in 35 participants, and so they were excluded from the per-protocol analysis (appendix p 10).

In the mITT population of suspected stroke, 145 (57%) of 254 participants in the nerinetide group and 147 (58%) of 253 in the placebo group had the primary outcome of a favourable functional outcome using the prespecified sliding dichotomy at 90 days. Using the prespecified primary analysis, adjusting for emergency medical services hub, age, and baseline LAMS score, there was no difference between the groups (adjusted odds ratio 1·05, 95% CI 0·73–1·51; adjusted risk ratio 1·04, 95% CI 0·85–1·25). Because there was no significant difference, all subsequent analyses in the mITT population, per-protocol population, those with a final diagnosis of acute ischaemic stroke or intracerebral haemorrhage, and those who received a reperfusion therapy (thrombolysis or endovascular thrombectomy, or both) were exploratory.

Despite the imbalance in stroke severity between the nerinetide and placebo groups that favoured placebo (tables 1 and 2), treatment with nerinetide was associated with numerically improved outcomes (table 3) in the mITT and per-protocol populations, in the acute ischaemic stroke group, and in those receiving reperfusion therapy. This finding was most pronounced in participants receiving reperfusion therapies (thrombolysis or endovascular thrombectomy, or both), and was consistent among the various clinical outcome measures including the responder definition selected as the preplanned primary outcome.

	Nerinetide		Placebo	
	n (%)	NIHSS	n (%)	NIHSS
Modified intention-to-treat population	254 (100%)	12 (5–19)	253 (100%)	10 (4–18)
Acute ischaemic stroke	172 (68%)	14 (7–19)	149 (59%)	10 (4–18)
Intracerebral haemorrhage	38 (15%)	19 (10–22)	55 (22%)	18 (10–22)
Any reperfusion	129 (51%)	16 (9–20)	104 (41%)	14 (7–18)
Thrombolysis	116 (46%)	16 (9–20)	96 (38%)	12 (6–18)
Endovascular thrombectomy	53 (21%)	18 (15–22)	45 (18%)	16 (13–19)
Acute ischaemic stroke with no reperfusion	43 (17%)	5 (3–14)	46 (18%)	4 (3–9)
Transient ischaemic attack	22 (9%)	2 (0–6)	22 (9%)	2 (0–4)
Mimic or other	22 (9%)	4 (2–10)	27 (11%)	2 (1–8)

Data are n (%) or median (IQR). NIHSS=National Institutes of Health Stroke Scale.

Table 2: **Participant numbers and baseline median stroke severities on emergency department arrival (NIHSS) in the modified intention-to-treat and exploratory populations**

There was a shift to improved functional outcome with nerinetide in the mITT population (proportional odds model; common odds ratio 1·54, 95% CI 1·09–2·17; figure 2A), the acute ischaemic stroke population overall (1·72, 1·31–2·60; figure 2B), and the acute ischaemic stroke subset that received any reperfusion therapy (2·13, 1·30–3·48; and the per-protocol population, 1·44, 1·00–2·08; figure 2C; table 3). By contrast, treatment with nerinetide was not associated with improved outcomes in participants with intracerebral haemorrhage, or in those with acute ischaemic stroke who did not subsequently receive a reperfusion therapy (table 3; appendix pp 28–29). These results are supported by adjusted analyses imputed for missing outcomes (appendix p 20) and with unadjusted analyses (appendix p 21).

Other notable outcomes, including worsening of stroke and mRS score of 0–1, also strongly favoured the nerinetide group in participants with acute ischaemic stroke, especially those who received a reperfusion therapy (table 3). This finding was corroborated by the infarct volumes, measured at 24 h or more after random assignment in participants that received any reperfusion therapy. Infarct volumes were lower in the nerinetide group (mean 27·7 mL, SD 44·4, median 9·6 mL, IQR 5·0–27·4) than the placebo group (mean 41·0 mL, SD 60·8, median 12·7 mL, IQR 4·3–49·3; adjusted odds ratio adjusted for age and NIHSS 0·66; 95% CI 0·46–0·95).

The safety population included all participants who received any amount of study drug (n=532). There were no differences in serious (table 4) or total adverse events (appendix pp 22–23) between groups, and nerinetide did not increase mortality or worsen stroke in any population (table 3; appendix p 21). The independent safety reviews

	Group 1			Group 2		
	N	Adjusted odds ratio (95% CI)	Adjusted risk ratio (95% CI)	N	Adjusted odds ratio (95% CI)	Adjusted risk ratio (95% CI)
Modified intention-to-treat population (group 1) and acute ischaemic stroke population (group 2)						
mRS responder	437*	1·41 (0·92 to 2·14)*	1·16 (0·97 to 1·39)*	302†	1·53 (0·93 to 2·52)†	1·21 (0·97 to 1·52)†
mRS shift (common odds ratio)	437*	1·54 (1·09 to 2·17)*	..	302†	1·72 (1·13 to 2·60)†	..
Worsening of stroke	437*	0·49 (0·26 to 0·94)*	0·61 (0·38 to 0·96)*	302†	0·43 (0·20 to 0·93)†	0·52 (0·28 to 0·94)†
Mortality	436*	0·64 (0·34 to 1·18)*	0·77 (0·54 to 1·10)*	301†	0·54 (0·25 to 1·14)†	0·66 (0·41 to 1·08)†
mRS 0–1	437*	1·57 (1·02 to 2·42)*	1·27 (1·01 to 1·59)*	302†	1·87 (1·12 to 3·14)†	1·42 (1·06 to 1·89)†
Barthel index score ≥95	437*	1·27 (0·83 to 1·96)*	1·01 (0·93 to 1·29)*	302†	1·39 (0·84 to 2·30)†	1·13 (0·93 to 1·38)†
EQ-5D-5L VAS (adjusted difference)	412*	12·68 (5·07 to 20·29)*	..	279†	15·22 (6·24 to 24·21)†	..
EQ-5D-5L index (adjusted difference)	422*	0·04 (–0·06 to 0·14)*	..	287†	0·07 (–0·06 to 0·21)†	..
Any reperfusion (group 1) and thrombolytics (group 2)						
mRS responder	222‡	1·84 (1·03 to 3·28)‡	1·29 (1·01 to 1·65)‡	203§	2·00 (1·07 to 3·73)§	1·34 (1·03 to 1·74)§
mRS shift (common odds ratio)	222‡	2·13 (1·30 to 3·48)‡	..	203§	2·38 (1·41 to 4·00)§	..
Worsening of stroke	222‡	0·33 (0·13 to 0·81)‡	0·40 (0·19 to 0·85)‡	203§	0·25 (0·10 to 0·72)§	0·32 (0·14 to 0·74)§
Mortality	221‡	0·48 (0·20 to 1·13)‡	0·60 (0·34 to 1·08)‡	202§	0·36 (0·14 to 0·93)§	0·51 (0·28 to 0·95)§
mRS 0–1	222‡	2·25 (1·24 to 4·10)‡	1·54 (1·11 to 2·12)‡	203§	2·30 (1·22 to 4·32)§	1·56 (1·10 to 2·19)§
Barthel index score ≥95	222‡	1·51 (0·84 to 2·72)‡	1·17 (0·94 to 1·46)‡	203§	1·75 (0·93 to 3·31)§	1·23 (0·97 to 1·55)§
EQ-5D-5L VAS (adjusted difference)	209‡	12·05 (1·16 to 22·94)‡	..	192§	15·78 (4·88 to 26·69)§	..
EQ-5D-5L index (adjusted difference)	214‡	0·06 (–0·08 to 0·20)‡	..	196§	0·10 (–0·05 to 0·25)§	..
Received mechanical thrombectomy (group 1) and acute ischaemic stroke without reperfusion (group 2)						
mRS responder	91¶	1·75 (0·70 to 4·38)¶	1·26 (0·87 to 1·82)¶	82‖	1·10 (0·36 to 3·38)‖	1·04 (0·67 to 1·60)‖
mRS shift (common odds ratio)	91¶	2·12 (0·96 to 4·68)¶	..	82‖	1·00 (0·44 to 2·28)‖	..
Worsening of stroke	91¶	0·75 (0·21 to 2·68)¶	0·78 (0·26 to 2·33)¶	82‖	0·94 (0·15 to 5·91)‖	0·97 (0·39 to 2·42)‖
Mortality	91¶	0·75 (0·22 to 2·58)¶	0·82 (0·35 to 1·93)¶	82‖	0·64 (0·12 to 3·50)‖	0·79 (0·33 to 1·93)‖
mRS 0–1	91¶	2·31 (0·91 to 5·83)¶	1·59 (0·95 to 2·67)¶	82‖	1·21 (0·37 to 3·91)‖	1·00 (0·64 to 1·85)‖
Barthel index score ≥95	91¶	1·10 (0·44 to 2·75)¶	1·03 (0·74 to 1·44)¶	82‖	1·29 (0·42 to 3·99)‖	1·08 (0·76 to 1·55)‖
EQ-5D-5L VAS (adjusted difference)	86¶	10·41 (–9·53 to 30·34)¶	..	72‖	12·51 (0·86 to 24·16)‖	..
EQ-5D-5L index (adjusted difference)	90¶	0·14 (–0·14 to 0·41)¶	..	75‖	–0·0040 (–0·14 to 0·13)‖	..
Intracerebral haemorrhage (group 1) and per-protocol population (group 2)						
mRS responder	74**	1·10 (0·21 to 5·66)**	1·04 (0·54 to 1·99)**	394††	1·21 (0·78 to 1·88)††	1·08 (0·90 to 1·31)††
mRS shift (common odds ratio)	74**	0·62 (0·22 to 1·75)**	..	394††	1·44 (1·00 to 2·08)††	..
Worsening of stroke	74**	1·84 (0·45 to 7·59)**	1·31 (0·72 to 2·38)**	394††	0·48 (0·24 to 0·96)††	0·59 (0·36 to 0·97)††
Mortality	74**	2·65 (0·58 to 12·19)**	1·31 (0·89 to 1·93)**	393††	0·69 (0·36 to 1·32)††	0·82 (0·55 to 1·17)††
mRS 0–1	74**	0·23 (0·02 to 2·46)**	0·48 (0·15 to 1·62)**	394††	1·44 (0·92 to 2·27)††	1·22 (0·96 to 1·55)††
Barthel index score ≥95	74**	2·11 (0·35 to 12·75)**	1·34 (0·68 to 2·62)**	394††	1·24 (0·79 to 1·95)††	1·09 (0·91 to 1·29)††
EQ-5D-5L VAS (adjusted difference)	73**	–13·43 (–33·79 to 6·92)**	..	374††	13·05 (5·39 to 20·71)††	..
EQ-5D-5L index (adjusted difference)	46**	0·04 (–0·11 to 0·19)**	..	318††	0·02 (–0·06 to 0·09)††	..

Binary logistic regression was used to derive adjusted odds ratios and risk ratios. These were adjusted for age and baseline NIHSS score in the emergency department and are presented with their 95% CIs. mRS shift analysis was conducted using an ordinal logistic regression and is reported as a common odds ratio for improved functional outcome, adjusted for age and NIHSS score in the emergency department. EQ-5D-5L index and VAS scores in treatment and control groups were compared using quantile regressions for multivariable analyses, adjusted for age and NIHSS score. Any reperfusion included the population receiving thrombolysis or mechanical thrombectomy or both. Thrombolytics included the population that received intravenous thrombolysis. EQ-5D-5L=European Quality of Life 5 Dimensions 5 Level version. EQ-5D-5L VAS= European Quality of Life 5 Dimensions 5 Level version Visual Analog Scale. mRS=modified Rankin Scale. NIHSS=National Institutes of Health Stroke Scale score. *Modified intention-to-treat population. †Acute ischaemic stroke population. ‡Any reperfusion. §Thrombolytics. ¶Received mechanical thrombectomy. ‖Acute ischaemic stroke without reperfusion. **Intracerebral haemorrhage. ††Per-protocol population.

Table 3: **Outcome by suspected stroke subpopulation (adjusted for age and NIHSS) for nerinetide *vs* placebo**

conducted when 25, 50, and 300 participants were enrolled into the trial recommended that the trial continue as planned.

Discussion

Nerinetide was not effective in reducing disability in all participants with clinically suspected stroke treated within 3 h of symptom onset. However, among participants with the target disease of acute ischaemic stroke, nerinetide treatment was associated with better outcomes at 90 days compared with placebo. This finding is consistent with the mechanism of action of nerinetide, an anti-ischaemic agent.[7,10] The mITT population included substantial numbers of participants with

intracerebral haemorrhage or stroke-mimicking conditions who were hypothesised to not benefit from nerinetide. This reduced the statistical power to show a benefit in the mITT population.

The key advantages of the FRONTIER trial were the speed of drug administration after symptom onset; the safety of nerinetide; and the absence of interference with in-hospital, time-sensitive, standard of care treatments such as thrombolysis or endovascular thrombectomy, or both. Dosing in the ambulance enabled the initiation of neuroprotection as soon as possible within the 3 h window. The safety of nerinetide was assessed in patients with a range of final diagnoses in addition to acute ischaemic stroke including transient ischaemic attack, intracerebral haemorrhage, and various stroke-mimicking conditions.

A further advantage was that the FRONTIER design eliminated the possibility of a drug–drug interaction between nerinetide and plasmin, after administration of tissue plasminogen activators. Plasmin cleaves and inactivates nerinetide if it is already in the circulation at the time nerinetide is given.[25,26] In FRONTIER, nerinetide was always administered before thrombolysis, because a thrombolytic could only be given later in hospital. Nerinetide has a short plasma half-life of 5–10 min[26] after which it enters into its target tissues where it is protected from cleavage and maintains its activity. Administering nerinetide before thrombolysis, as in this study, avoids its inactivation.

Additionally, the trial enrolled an embedded negative control by including subpopulations in which no treatment benefit is expected from an anti-ischaemic agent (eg, intracerebral haemorrhage). A treatment benefit in participants with acute ischaemic stroke is less likely due to chance if it is not also observed in participants for whom a benefit is not expected. The trial enrolled a high proportion of Asian participants, making its results more generalisable to important stroke populations.

From a physiological perspective, among participants with a final diagnosis of acute ischaemic stroke, the trial probably enrolled both slow and rapid stroke progressors.[27] Rapid progressors are typically individuals with poor leptomeningeal collaterals who, by the time of late treatment windows for endovascular thrombectomy trials (up to 24 h[28,29]), might no longer have salvageable brain tissue.[27] By enrolling in a 3 h window, rapid progressors, who might benefit the most from an agent that slows stroke progression, might have a clinical benefit from neuroprotection. In primate models of middle cerebral artery occlusion with treatment with nerinetide at 60 min from stroke onset, the largest effect size is observed in occlusions that result in poor collaterals.[10] Unlike modern endovascular thrombectomy trials,[28,29] FRONTIER did not exclude participants based on medical imaging that might eliminate those with rapid stroke progression.

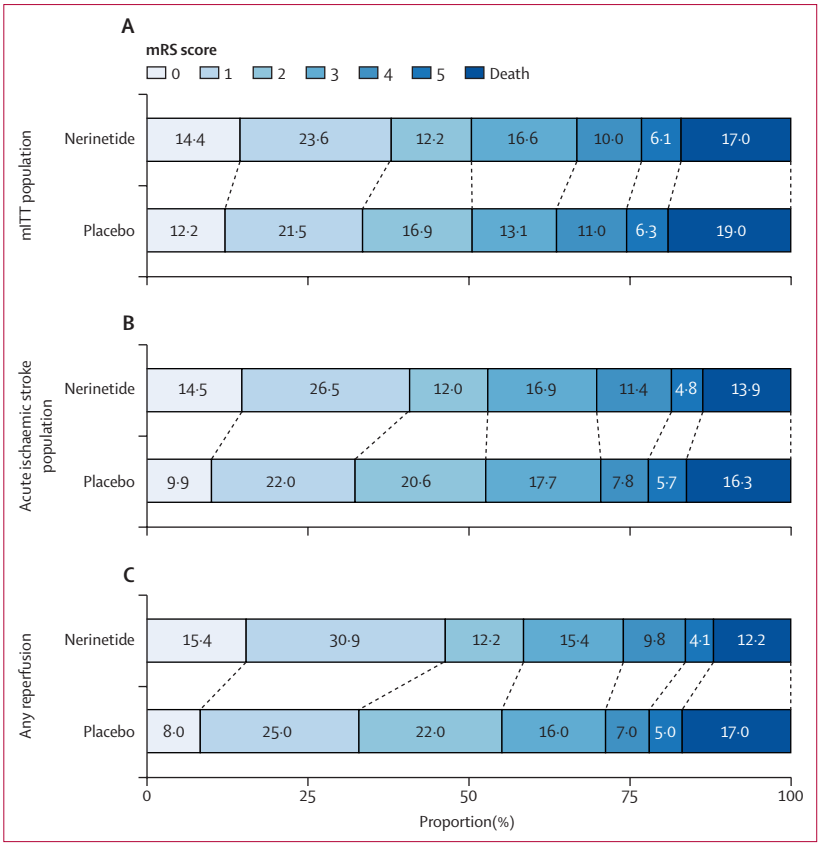

Figure 2: **Day 90 mRS distribution by nerinetide and placebo groups**
Analyses were conducted using a proportional odds model adjusted for age and baseline NIHSS across the mRS scale. (A) mITT population (n=466; common OR: 1·54 [95% CI 1·09–2·17]; p=0·015). (B) Acute ischaemic stroke population (n=307; common OR 1·72 [95% CI 1·31–2·60]; p=0·011). (C) Any reperfusion population (n=223; common OR: 2·13 [95% CI 1·30–3·48]; p=0·003). mITT=modified intention-to-treat. mRS=modified Rankin Scale. NIHSS=National Institutes of Health Stroke Scale. OR=odds ratio.

	Nerinetide (n=265)	Placebo (n=267)	Total (n=532)
Number of serious treatment-emergent adverse events	121	111	232
Participants with at least one serious treatment-emergent adverse event*	93 (35%)	97 (36%)	190 (36%)
Nervous system disorders	30 (11%)	40 (15%)	70 (13%)
Stroke in evolution	6 (2%)	5 (2%)	11 (2%)
Haemorrhagic transformation stroke	4 (2%)	6 (2%)	10 (2%)
Ischaemic stroke	4 (2%)	0	4 (1%)
Carotid artery stenosis	3 (1%)	0	3 (1%)
Seizure	12 (5%)	9 (3%)	21 (4%)
Respiratory, thoracic, and mediastinal disorders	4 (2%)	0	4 (1%)
Pulmonary embolism	3 (1%)	3 (1%)	6 (1%)
Aspiration	4 (2%)	2 (1%)	6 (1%)
Psychiatric disorders	3 (1%)	0	3 (1%)
Delirium	93 (35%)	97 (36%)	190 (36%)

Data are n (%). Most common serious treatment-emergent adverse events (defined as an adverse event that began after the start of trial medication) had a frequency of more than 1% in the nerinetide group. The safety population includes patients who received any dose of study drug (n=532). MedDRA=Medical Dictionary for Regulatory Activities. *There were no differences in the frequency of serious treatment-emergent adverse events between treatment groups, p=0·7865.

Table 4: **Serious treatment-emergent adverse events**

Similar to other recent stroke trials of prehospital interventions,[20,30–33] FRONTIER showed the feasibility of administering a pharmacological stroke treatment by paramedics early after symptom onset. It is complementary to the neutral RIGHT-2[30] and MR-ASAP[31] studies, which tested an agent that increased circulating nitric oxide levels, in that nerinetide blocks nitric oxide production in neurons. The FRONTIER intervention was provided by paramedics in high functioning paramedic services with strong medical oversight. They delivered a drug that must be cooled in custom fridges, dosed by weight, and delivered by a pump over 10 min.

Participants arrived at the stroke centre shortly after initiation of the intervention and many received thrombolysis or endovascular thrombectomy, or both. The incremental benefit of nerinetide for patients with acute ischaemic stroke suggested in this study implies that even with access to timely initiation of reperfusion, adequate recanalisation takes time to achieve and slowing stroke progression in this interval might be of benefit. More recently, mobile stroke units (MSUs) have enhanced access to thrombolysis early after stroke onset.[34] MSUs have allowed for the clearer identification of the target population by providing imaging in the field, allowing for greater statistical efficiency of MSU-based treatment trials. Given that thrombolysis is a chemical process that might take hours to complete, nerinetide might be of benefit in MSUs, although it should be administered ahead of the thrombolytic. It also might be that use of an effective neuroprotectant in the potential thrombolytic population is a more cost-effective approach than MSUs with CT scanners. Further investigation into the effectiveness of early neuroprotection in communities far removed from stroke centres will be needed.

The benefit of nerinetide in a suspected stroke population should be balanced with potential harms. Similar numbers of serious adverse events occurred in both the nerinetide and placebo groups. At high doses in animals, nerinetide causes a transient elevation of circulating histamine, released via mast cell degranulation. This effect could cause reactions such as hypotension, flushing, urticaria, and pruritus. Although numerically more instances of transient hypotension occurred with nerinetide than with placebo, those instances were mild and self-limiting. The nerinetide group had numerically fewer instances of worsening of stroke than the placebo group. As used in this trial, nerinetide was well tolerated, with no medically meaningful differences in adverse events from placebo. In particular, the intracerebral haemorrhage population did not witness worse outcomes or increased adverse events. This safety profile suggests the acceptable safety of nerinetide when it is given before imaging confirmation of acute ischaemic stroke and thus indicates its suitability for the prehospital setting.

The results of FRONTIER, including in the endovascular thrombectomy subpopulation, are consistent with the results of ESCAPE-NA1[25] and ESCAPE-NEXT[35] in participants enrolled in the first 3 h after symptom onset.[36] These data support the further exploration of neuroprotection within this crucial early time window.

This trial had notable limitations. The LAMS in this study did not correlate with outcome and was of little use when adjusting for differences in baseline stroke severity between nerinetide and placebo groups. Ultimately, there was an imbalance in stroke severity between the nerinetide and placebo groups. Thus, the LAMS was used in the prespecified primary analysis because evaluation of the NIHSS in the field was out of scope for paramedics. The first NIHSS was a post-randomisation variable obtained on emergency department arrival. However, given the exploratory nature of the analyses, we felt it crucial to adjust for stroke severity in the emergency department owing to the importance of this prognostic covariate.[21,22] A hypothesis that we cannot test directly given the current dataset is that nerinetide adversely affected NIHSS to cause this imbalance. However, this is unlikely because this agent is non-sedating and has no known biological reason to accelerate stroke progression, especially in the short interval from dosing to emergency department arrival (median of 11 min; table 1). If this had been a drug effect rather than random variation, then a similar imbalance would have also been observed in participants with transient ischaemic attack and intracerebral haemorrhage, but this was not the case (table 2). Given that each ambulance was stocked with only one study drug vial, it was impossible to incorporate the minimisation into the random assignment process to reduce imbalances. Another key limitation was that the study enrolled substantial numbers of participants without the target diagnosis of acute ischaemic stroke, reducing the power of the overall design to detect a treatment effect in the mITT population.

In conclusion, prehospital administration of nerinetide did not show neuroprotection in all participants with suspected stroke treated within 3 h of symptom onset. Adjusting for disparities in age and stroke severity revealed that nerinetide might benefit patients with acute ischaemic stroke, selected for reperfusion therapies within 3 h of symptom onset. This finding will require confirmation.

Contributors

The manuscript was written by the Publication Committee, which included JC, MDH, RHS, and MT. JC, MDH, RHS, and MT took the primary responsibility for crafting the manuscript text and provided the overall principal leadership for the study. JC: conceptualisation, funding acquisition, investigation, methodology, project administration, supervision, original draft, and review and editing.
MDH: conceptualisation, data curation, formal analysis, methodology, validation, original draft, and review and editing. RHS: conceptualisation, methodology, investigation, original draft, and review and editing. CA: formal analysis, and review and editing. OB: investigation and review and editing. LKC: investigation and review and editing. SC: investigation, supervision, and review and editing. AG: data curation, and review and editing. JDG: conceptualisation, funding acquisition, methodology, and

review and editing. CH: project administration, supervision, and review and editing. DRH: conceptualisation, investigation, project administration, and review and editing. KH: project administration, and review and editing. SJ: investigation, project administration, and review and editing. YK: data curation, formal analysis, and review and editing. ML: project administration, supervision, and review and editing. DM-N: formal analysis. GM: investigation and review and editing. MM: investigation and review and editing. LJM: conceptualisation, funding acquisition, investigation, and review and editing. JMO: formal analysis. SP: investigation, project administration, and review and editing. YP: investigation and review and editing. DS: investigation and review and editing. AS: formal analysis. JT: project administration, investigation, and review and editing. AT: investigation, and review and editing. PRV: investigation, supervision, and review and editing. MT: conceptualisation, data curation, formal analysis, funding acquisition, methodology, resources, supervision, validation, original draft, and review and editing. MT and MDH accessed and verified the data.

Declaration of interests

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Data sharing

Patient-level data from the FRONTIER study are not currently publicly available. Our plan is to make the anonymised data publicly available in the future. The timing of this availability has not been established. Criteria for gaining access and location of the data will be established at a future date.

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Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Christenson J, Hill MD, Swartz RH, et al. Efficacy and safety of intravenous nerinetide initiated by paramedics in the field for acute cerebral ischaemia within 3 h of symptom onset (FRONTIER): a phase 2, multicentre, randomised, double-blind, placebo-controlled study. *Lancet* 2025; **405:** 571–82.

Supplementary Appendix

Table of Contents

ACKNOWLEDGEMENTS: SITES AND INVESTIGATORS

Trial leadership and management:

Trial Executive Committee:

NoNO Inc: Michael Tymianski, Kathy J Heard, Michelle Leroux, Cameron Harris, J David Garman

Coordinating Investigator/Staff: Jim Christenson MD, Richard H. Swartz MD, Michael Hill MD, Sarah Pennington

Academic Investigators: Devin Harris MD, Sandra Jenneson MD, Aleks Tkach MD, George Medvedev MD, Oscar Benavente MD

Funding: Brain Canada and NoNO Inc.

Regulatory sponsor: NoNO Inc.

Michael Tymianski MD, PhD, Alphonse Potvin, Cameron Harris, Corey Adams PhD, Diana Mayor PhD, J David Garman PhD, Davis Chau, Hartleigh Bailey, Kathy J Heard, Liat Bermanis, Lucy Teves, Maria Galsim, Michal Helbin, Michelle Leroux, Nilima Bendre, Olenka Doppelt, Sandra Cuthbert, Yichuan Zhang, Yatika Kohli PhD, Yvonne Garcia, Michael Salter MD, PhD, Cathy Metcalfe, Sue Bonar, Katharine Armitage, Frieder Hofmann PhD, John Constant PhD, Monica Bologa MD, Aravind Ganesh, Roberta, Anderson PhD, Julie Bishop

Under the oversight of NoNO Inc, Data Management was provided by Clario USA (previously known as Bioclinica) and Statistics, as defined in the statistical analysis plan, were provided by Allucent USA (formerly Cato Research Canada, Inc.).

Statement of Trial Organization

The FRONTIER trial was designed by the academic investigators (University of British Columbia [JC], University of Calgary [MDH], University of Toronto [RS, MT]) and the sponsor (NoNO, Toronto, ON, Canada). The data were gathered by the sites, the trial was coordinated at the University of British Columbia. A trial executive committee comprised of the academic investigators and representatives from NoNO Inc (see above) was responsible for the overall running of the trial. The trial was funded by grants from NoNO Inc to Brain Canada and by NoNO Inc.

The data were gathered by the sites, who were responsible for the recruitment, data collection patient flow, quality of care. NoNO Inc provided regulatory oversight, site support, monitoring, data management, biostatistics support. Drug manufacture, shipping and management was contracted to the University of Iowa.

The decision to publish was made by the academic investigators and the sponsor jointly. The manuscript was written by the Publication Committee (JC, MDH, RS and MT). All took the primary responsibility for crafting the manuscript text and provided the overall principal leadership for the study. All authors provided critical revisions to the manuscript text. Declaration of interests. The paper was written by the Publication Committee, which included one member from the sponsor. Confidentiality agreements were in place between the authors and sponsor. All the authors vouch for the accuracy and completeness of the data and data analyses and for the fidelity of this report to the study protocol and the complete reporting of adverse events.

The trial consisted of a 'Pre-hospital' phase (Ambulance) and an 'In-hospital' phase. In total, 10 qualified Investigators were involved in the trial; one at each emergency medical services (EMS) hub in Ontario, one for both EMS hubs in British Columbia, and one at each hospital. In the Pre-hospital phase, a total of 3 EMS (i.e., ambulance services) enrolled and administered study drug and transported trial participants to 7 trial centres. For the In-hospital phase, the trial centres were hospitals that were thrombolysis-capable stroke centres qualified to treat stroke patients according to contemporaneous medical guidelines.

Ambulance Hubs and Trial Sites/Hospitals

Paramedic Sites	Trial Site/ Hospital
Toronto Paramedic Services	Site 11/St. Michael's Hospital
	Site 12/Sunnybrook Health Sciences
	Site 13/Toronto Western Hospital
Peel Regional Paramedic Services	Site 14/Trillium Health Partners
BC Emergency Health Services	Site 15/Vancouver General Hospital
	Site 16/ Royal Columbian Hospital
	Site 17/Kelowna General Hospital

Coordinating Centre: British Columbia
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Coordinating Investigator: Jim Christenson MD
Contributors: Sarah Pennington, Christi Sawyer, Vishaya Naidoo

Coordinating Centre: Ontario
Sunnybrook Health Sciences Centre, University of Toronto, Toronto ON, Canada
Coordinating Investigator /Site PI: Richard H. Swartz MD PhD
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Site Investigator: Manu Mehdiratta MD

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SUPPLEMENTAL METHODS

Preparation of Nerinetide (NA-1)

- Nerinetide and placebo were identical in appearance. All vials were labelled with a unique vial number. Vials were stored at 2-8º C. Each vial of nerinetide contained 13.5 ml of solution to a maximum of 270 mg of nerinetide.
- The weight-adjusted volume was drawn up in a standard syringe and injected into a 50 mL drip bag of 0.9% normal saline. Study drug was administered over 10 (±1) minutes using an infusion pump.

Consent

Specific requirements followed by Canadian ethics boards for approving prehospital emergency research studies using waiver of consent are described in the Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans (https://ethics.gc.ca/eng/policy-politique_tcps2-eptc2_2022.html). This is a joint policy of Canada's three federal research agencies – the Canadian Institutes of Health Research (CIHR), the Natural Sciences and Engineering Research Council of Canada (NSERC), and the Social Sciences and Humanities Research Council (SSHRC). The FRONTIER study met all criteria to allow waiver of consent. Specific details on the consent process are provided in Section 6.3.2 of the study Protocol.

Sliding Dichotomy Definition of Responder at Day 90 Follow Up

	Prehospital LAMS 2-3	**Prehospital LAMS 4-5**
Age 79 or under	mRS 0-1	mRS 0-2
Age 80 or over	mRS 0-2	mRS 0-2

LAMS = Los Angeles Motor Score

CONSORT CHECKLIST

CONSORT 2010 checklist of information to included when reporting a randomised trial

Section/ Topic	Item No	Checklist Item	Reported
Title and abstract			
	1a	Identification as a randomized trial in the title	yes
	1b	Structured summary of trial design, methods, results, and conclusions	yes
Introduction			
Background and	2a	Scientific background and explanation of rationale	yes
Objectives	2b	Specific objectives or hypothesis	yes
Methods			
Trial design	3a	Description of trial design including allocation ratio	yes
	3b	Important changes to methods after trial commencement with reasons	yes
Participants	4a	Eligibility criteria for participants	Table S18
	4b	Settings and locations where the data were collected	yes
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	yes
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	yes
	6b	Any changes to trial outcomes after the trial commenced, with reasons	yes
Sample size	7a	How sample size was determined	yes
	7b	When applicable, explanation of any interim analysis and stopping guidelines	yes
Randomization:	8a	Method used to generate the random allocation sequence	yes
Seqeunce generation	8b	Type of randomisation; details of any restrictions	yes
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence, describing any steps taken to conceal the sequence until interventions were assigned	yes
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	yes
Blinding	11a	If done, who was blinded after assignment to interventions and how	yes
	11b	If relevant, description of the similarity of interventions	yes
Statistical Methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	yes
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	yes
Results			
Participant flow	13a	For each age group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	Table 1, Figure 1
	13b	For each group, losses and exclusions after randomisation, together with reasons	Figure 1, Table S4
Recruitment	14a	Dates defining the periods of recruitment and follow-up	yes
	13b	Why the trial ended or was stopped	yes
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	Table1, S5 – S10
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	Table 3
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% CI)	Table 3, Figure 2
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	Table 3, Table S15
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	Table 3, Table S2, Table S3, Table S14, Table S15, Figure S1-S5
Harms	19	All important harms or unintended effects in each group	Table 4, Table S16, Table S17
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	yes
Generalisability	21	Generalisability of the trial findings	yes
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	yes
Other Information			
Registration	23	Registration number and name of trial registry	yes
Protocol	24	Where the full trial protocol can be accessed	Supplementary Appendix
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	yes

SUPPLEMENTARY TABLES AND FIGURES

Table S1: Frequency of participants who declined or withdrew consent in FRONTIER

Treatment Groups	Participant Disposition [n (%)]	
Nerinetide	11 (44.0%)	
Placebo	14 (56.0%)	
Total	25 (100%)	
Pearson chi2	2.6786	p = 0.102
Fisher's exact		p = 0.230
1-sided Fisher's exact		p = 0.158

Table S2: Overall rate of imputation of mRS day 90 outcomes in FRONTIER (mITT Population)

Treatment Groups	mRS Day 90 Outcomes		Total [n (%)]
	Not Missing [n (%)]	Missing and Imputed[1] [n (%)]	
Nerinetide	229 (49.14%)	25 (60.98%)	254 (50.1%)
Placebo	237 (50.86%)	16 (39.02%)	253 (49.9%)
Total	466 (100%)	41 (100%)	507 (100%)
Pearson chi[2]	2.6786	p = 0.146	
Fisher's exact		p = 0.192	
1-sided Fisher's exact		p = 0.098	

[1]Single imputation of missing data was performed according to the rules in the statistical analysis plan.

Table S3: Rate of imputation of mRS day 90 outcomes in FRONTIER by discharge diagnosis (mITT Population)

mRS Day 90 Outcomes	Discharge Diagnosis [n (%)]					Total [n (%)]
	Haemorrhagic Stroke	Ischemic Stroke	Other	TIA	Un-determined	
Not Missing	86 (92.47)	307 (95.64)	30 (68.18)	39(88.64)	4 (80.00)	466 (91.91)
Missing and Imputed[1]	7 (7.53)	14 (4.36)	14 (31.82)	5 (11.36)	1 (20.00)	41 (8.09)
Total	93 (100)	321 (100)	44 (100)	44 (100)	5 (100)	507 (100)
Pearson chi2	40.9618	p = 0.000				
Fisher's exact		p = 0.000				

[1]Single imputation of missing data was performed according to the rules in the statistical analysis plan. There were significant differences in the rates of missing outcomes (imputation) between the groups.

Table S4: Major Protocol Deviations in FRONTIER (mITT Population)

Randomization Variable	Nerinetide (N = 254) [n (%)]	Placebo (N = 253) [n (%)]	Total (N = 507) [n (%)]
Participants Excluded from Per protocol population	17 (6.7)	18 (7.1)	35 (6.9)
Inclusion Criteria	7 (2.8)	7 (2.8)	14 (2.8)
Exclusion Criteria	3 (1.2)	0	3 (0.6)
Study Drug Dosing - Dose Volume Not Compliant	4 (1.6)	7 (2.8)	11 (2.2)
Study Drug Dosing - Infusion Out of Window	3 (1.2)	4 (1.6)	7 (1.4)

A total of 35 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 (17 participants, 6.7%) and placebo group (18 participants, 6.9%).

Table S5: Participant Demographics in FRONTIER (Safety Population)

	Nerinetide (N = 265)	**Placebo (N = 267)**	**Total (N = 532)**
Age (years; median [IQR])	73 [64, 81]	75 [64, 82]	74 [64, 82]
Sex [n (%)]			
Men	155 (58.5)	147 (55.1)	302 (56.8)
Women	110 (41.5)	120 (44.9)	230 (43.2)
Race [n (%)]			
White	166 (62.6)	166 (62.2)	332 (62.4)
Black or African American	4 (1.5)	7 (2.6)	11 (2.1)
Asian	86 (32.5)	86 (32.2)	172 (32.3)
Other	9 (3.4)	8 (3.0)	17 (3.2)
Medical History [n (%)]			
Cardiovascular	163 (61.5)	155 (58.1)	318 (59.8)
Congestive heart failure or pulmonary edema	22 (8.3)	30 (11.2)	52 (9.8)
Peripheral vascular disease	17 (6.4)	30 (11.2)	47 (8.8)
Vascular Risk Factors			
Hypertension	193 (72.8)	190 (71.2)	383 (72.0)
Diabetes mellitus	67 (25.3)	60 (22.5)	127 (23.9)
Hyperlipidemia	129 (48.7)	129 (48.3)	258 (48.5)
Atrial fibrillation	76 (28.7)	69 (25.8)	145 (27.3)
Tobacco (current or within past year)	46 (17.4)	47 (17.6)	93 (17.5)
CABG/Coronary angioplasty	29 (10.9)	27 (10.1)	56 (10.5)
Prior cerebral infarct(s)	77 (29.1)	50 (18.7)	127 (23.9)
Prior TIA(s)	40 (15.1)	29 (10.9)	69 (13.0)
Prior intracerebral hemorrhage(s)	9 (3.4)	8 (3.0)	17 (3.2)
Prior stroke, infarct vs. hemorrhage unknown	23 (8.7)	18 (6.7)	41 (7.7)
Renal disease	39 (14.7)	34 (12.7)	73 (13.7)
Dialysis	1 (0.4)	0	1 (0.2)
Existing hemiplegia or paraplegia	8 (3.0)	10 (3.7)	18 (3.4)
Clinical Characteristics & Workflow			
Type of Stroke [n (%)]			
Hemorrhagic (ICH)	38 (14.3)	57 (21.3)	95 (17.9)
Ischemic	181 (68.3)	155 (58.1)	336 (63.2)
TIA	22 (8.3)	22 (8.2)	44 (8.3)
Stroke Mimic/Undetermined	24 (9.1)	33 (12.4)	57 (10.7)
Stroke Severity (Median [IQR])			
Baseline LAMS	4 [3, 5]	4 [3, 5]	4 [3, 5]
ED Arrival NIHSS	12 [5, 19]	9.5 [4, 18]	11 [5, 18]
Reperfusion Received [n (%)]			
Intravenous thrombolysis (IVT)	123 (46.4)	99 (37.1)	222 (41.7)
Endovascular Thrombectomy (EVT)	55 (20.8)	46 (17.2)	101 (19.0)
IVT and/or EVT (Any Reperfusion)	129 (48.7)	104 (39.0)	233 (43.8)
Workflows (Median [IQR])			
Last seen normal to dosing (minutes)	60 [45, 94]	66 [49, 106]	64 [46, 100]
Dosing to IVT*	55 [42, 70]	56 [43, 67]	55 [42, 68]
Dosing to EVT**	106 [78, 130]	97 [77, 130]	103 [77, 130]

*nerinetide n=123; placebo n = 99; ** nerinetide n=55; placebo n = 46
Safety Population, defined as all patients who received any amount of study drug.

Table S6: Participant Demographics of Acute Ischemic Stroke Population in FRONTIER

	Nerinetide (N = 172)	**Placebo (N = 149)**	**Total (N = 321)**
Age (years; median [IQR])	76 [67, 82]	76 [65, 83]	76 [66, 83]
Sex [n (%)]			
Men	97 (56.4)	83 (55.7)	180 (56.1)
Women	75 (43.6)	66 (44.3)	141 (43.9)
Race [n (%)]			
White	114 (66.3)	96 (64.4)	210 (65.4)
Black or African American	4 (2.3)	4 (2.7)	8 (2.5)
Asian	49 (28.5)	46 (30.9)	95 (29.6)
Other	5 (2.9)	3 (2.0)	8 (2.5)
Medical History [n (%)]			
Cardiovascular	115 (66.9)	104 (69.8)	219 (68.2)
Congestive heart failure or pulmonary edema	18 (10.5)	24 (16.1)	42 (13.1)
Peripheral vascular disease	13 (7.6)	22 (14.8)	35 (10.9)
Vascular Risk Factors			
Hypertension	129 (75)	108 (72.5)	237 (73.8)
Diabetes mellitus	48 (27.9)	29 (19.5)	77 (24.0)
Hyperlipidemia	84 (48.8)	78 (52.3)	162 (50.5)
Atrial fibrillation	55 (32.0)	49 (32.9)	104 (32.4)
Tobacco (current or within past year)	33 (19.2)	26 (17.4)	59 (18.4)
CABG/Coronary angioplasty	19 (11.0)	18 (12.1)	37 (11.5)
Prior cerebral infarct(s)	48 (27.9)	35 (23.5)	83 (25.9)
Prior TIA(s)	27 (15.7)	16 (10.7)	43 (13.4)
Prior intracerebral hemorrhage(s)	3 (1.7)	6 (4.0)	9 (2.8)
Prior stroke, infarct vs. hemorrhage unknown	14 (8.1)	10 (6.7)	24 (7.5)
Renal disease	25 (14.5)	21 (14.1)	46 (14.3)
Dialysis	1 (0.6)	0	1 (0.3)
Existing hemiplegia or paraplegia	0	5 (3.4)	5 (1.6)
Clinical Characteristics & Workflow			
Type of Stroke [n (%)]			
Hemorrhagic (ICH)	0	0	0
Ischemic	172 (100)	149 (100)	321 (100)
TIA	0	0	0
Stroke Mimic/Undetermined	0	0	0
Stroke Severity (Median [IQR])			
Baseline LAMS	4 [3, 5]	4 [3, 5]	4 [3, 5]
ED Arrival NIHSS	14 [7, 19]	10 [4, 18]	12 [6, 18]
Reperfusion Received [n (%)]			
Intravenous thrombolysis (IVT)	115 (66.9)	95 (63.8)	210 (65.4)
Endovascular Thrombectomy (EVT)	52 (30.2)	45 (30.2)	97 (30.2)
IVT and/or EVT (Any Reperfusion)	128 (74.4)	103 (69.1)	231 (72.0)
Workflows (Median [IQR])			
Last seen normal to dosing (minutes)	59.0 [44, 95]	70.0 [51, 112]	64.0 [48, 102]
Dosing to IVT*	54 [41, 69]	56 [43, 66]	55 [42, 67]
Dosing to EVT**	104 [77, 124]	95 [77, 131]	102 [77, 129]

*nerinetide n=115; placebo n = 95; ** nerinetide n=52; placebo n = 45

Table S7: Participant Demographics of Any Reperfusion Population in FRONTIER

	Nerinetide (N = 129)	**Placebo (N = 104)**	**Total (N = 233)**
Age (years; median [IQR])	75 [66, 82]	76 [63, 83]	76 [66, 82]
Sex [n (%)]			
Men	73 (56.6)	55 (52.9)	128 (54.9)
Women	56 (43.4)	49 (47.1)	105 (45.1)
Race [n (%)]			
White	89 (69.0)	66 (63.5)	155 (66.5)
Black or African American	2 (1.6)	2 (1.9)	4 (1.7)
Asian	33 (25.6)	32 (30.8)	65 (27.9)
Other	5 (3.9)	4 (3.8)	9 (3.9)
Medical History [n (%)]			
Cardiovascular	85 (65.9)	74 (71.2)	159 (68.2)
Congestive heart failure or pulmonary edema	13 (10.1)	16 (15.4)	29 (12.4)
Peripheral vascular disease	9 (7.0)	11 (10.6)	20 (8.6)
Vascular Risk Factors			
Hypertension	97 (75.2)	76 (73.1)	173 (74.2)
Diabetes mellitus	32 (24.8)	25 (24.0)	57 (24.5)
Hyperlipidemia	62 (48.1)	56 (53.8)	118 (50.6)
Atrial fibrillation	39 (30.2)	35 (33.7)	74 (31.8)
Tobacco (current or within past year)	24 (18.6)	17 (16.3)	41 (17.6)
CABG/Coronary angioplasty	16 (12.4)	14 (13.5)	30 (12.9)
Prior cerebral infarct(s)	30 (23.3)	21 (20.2)	51 (21.9)
Prior TIA(s)	18 (14.0)	11 (10.6)	29 (12.4)
Prior intracerebral hemorrhage(s)	1 (0.8)	4 (3.8)	5 (2.1)
Prior stroke, infarct vs. hemorrhage unknown	7 (5.4)	6 (5.8)	13 (5.6)
Renal disease	17 (13.2)	14 (13.5)	31 (13.3)
Dialysis	0	0	0
Existing hemiplegia or paraplegia	0	3 (2.9)	3 (1.3)
Clinical Characteristics & Workflow			
Type of Stroke [n (%)]			
Hemorrhagic (ICH)	0	0	0
Ischemic	128 (99.2)	103 (99.0)	231 (99.1)
TIA	1 (0.8)	0	1 (0.4)
Stroke Mimic/Undetermined	0	1 (1.0)	1 (0.4)
Stroke Severity (Median [IQR])			
Baseline LAMS	5 [3, 5]	4 [3, 5]	5 [3, 5]
ED Arrival NIHSS	16 [9, 20]	14 [7, 18]	14.5 [8, 19]
Reperfusion Received [n (%)]			
Intravenous thrombolysis (IVT)	116 (89.9)	96 (92.3)	212 (91.0)
Endovascular Thrombectomy (EVT)	52 (40.3)	45 (43.3)	97 (41.6)
IVT and/or EVT (Any Reperfusion)	129 (100)	104 (100)	233 (100)
Workflows (Median [IQR])			
Last seen normal to dosing (minutes)	55 [43, 77]	67 [50, 105]	60 [45, 90]
Dosing to IVT*	54 [41, 69]	56 [43, 66]	55 [42, 67]
Dosing to EVT**	104 [77, 124]	95 [77, 131]	102 [77, 129]

*nerinetide n=116; placebo n = 96; ** nerinetide n=52; placebo n = 45

Table S8: Participant Demographics Thrombolysis Population in FRONTIER

	Nerinetide (N = 116)	**Placebo (N = 96)**	**Total (N = 212)**
Age (years; median [IQR])	75 [66, 81]	76 [65, 83]	76 [66, 82]
Sex [n (%)]			
Men	66 (56.9)	50 (52.1)	116 (54.7)
Women	50 (43.1)	46 (47.9)	96 (45.3)
Race [n (%)]			
White	82 (70.7)	61 (63.5)	143 (67.5)
Black or African American	2 (1.7)	2 (2.1)	4 (1.9)
Asian	27 (23.3)	29 (30.2)	56 (26.4)
Other	5 (4.3)	4 (4.2)	9 (4.2)
Medical History [n (%)]			
Cardiovascular	76 (65.5)	67 (69.8)	143 (67.5)
Congestive heart failure or pulmonary edema	12 (10.3)	14 (14.6)	26 (12.3)
Peripheral vascular disease	9 (7.8)	10 (10.4)	19 (9.0)
Vascular Risk Factors			
Hypertension	87 (75.0)	71 (74.0)	158 (74.5)
Diabetes mellitus	26 (22.4)	25 (26.0)	51 (24.1)
Hyperlipidemia	52 (44.8)	51 (53.1)	103 (48.6)
Atrial fibrillation	31 (26.7)	33 (34.4)	64 (30.2)
Tobacco (current or within past year)	24 (20.7)	15 (15.6)	39 (18.4)
CABG/Coronary angioplasty	14 (12.1)	14 (14.6)	28 (13.2)
Prior cerebral infarct(s)	27 (23.3)	21 (21.9)	48 (22.6)
Prior TIA(s)	16 (13.8)	7 (7.3)	23 (10.8)
Prior intracerebral hemorrhage(s)	0	3 (3.1)	3 (1.4)
Prior stroke, infarct vs. hemorrhage unknown	6 (5.2)	6 (6.3)	12 (5.7)
Renal disease	15 (12.9)	12 (12.5)	27 (12.7)
Dialysis	0	0	0
Existing hemiplegia or paraplegia	0	3 (3.1)	3 (1.4)
Clinical Characteristics & Workflow			
Type of Stroke [n (%)]			
Hemorrhagic (ICH)	0	0	0
Ischemic	115 (99.1)	95 (99)	210 (99.1)
TIA	1 (0.9)	0	1 (0.5)
Stroke Mimic/Undetermined	0	1 (1.0)	1 (0.5)
Stroke Severity (Median [IQR])			
Baseline LAMS	5 [3, 5]	4 [3, 5]	4.5 [3, 5]
ED Arrival NIHSS	16 [9, 20]	12 [7, 18]	14 [8, 19]
Reperfusion Received [n (%)]			
Intravenous thrombolysis (IVT)	116 (100)	96 (100)	212 (100)
Endovascular Thrombectomy (EVT)	39 (33.6)	37 (38.5)	76 (35.8)
IVT and/or EVT (Any Reperfusion)	116 (100)	96 (100)	212 (100)
Workflows (Median [IQR])			
Last seen normal to dosing (minutes)	55 [42, 74]	65 [50, 102]	59 [45, 85]
Dosing to IVT*	54 [41, 69]	56 [43, 66]	55 [42, 67]
Dosing to EVT**	106 [81, 126]	99 [80, 132]	103 [79, 131]

*nerinetide n=116; placebo n = 96; ** nerinetide n=39; placebo n =37

Table S9: Participant Demographics of EVT Population in FRONTIER

	Nerinetide (N = 53)	**Placebo (N = 45)**	**Total (N = 98)**
Age (years; median [IQR])	72 [63, 85]	64 [56, 85]	72 [59, 85]
Sex [n (%)]			
Men	27 (10.6)	25 (9.9)	52 (10.3)
Women	26 (10.2)	20 (7.9)	46 (9.1)
Race [n (%)]			
White	38 (15)	28 (11.1)	66 (13.0)
Black or African American	0 (0)	1 (0.4)	1 (0.2)
Asian	14 (5.5)	14 (5.5)	28 (5.5)
Other	1 (0.4)	2 (0.8)	3 (0.6)
Medical History [n (%)]			
Cardiovascular	158 (62.2)	149 (58.9)	307 (60.6)
Congestive heart failure or pulmonary edema	22 (8.7)	28 (11.1)	50 (9.9)
Peripheral vascular disease	16 (6.3)	29 (11.5)	45 (8.9)
Vascular Risk Factors			
Hypertension	186 (73.2)	181 (71.5)	367 (72.4)
Diabetes mellitus	63 (24.8)	56 (22.1)	119 (23.5)
Hyperlipidemia	124 (48.8)	123 (48.6)	247 (48.7)
Atrial fibrillation	73 (28.7)	64 (25.3)	137 (27)
Tobacco (current or within past year)	45 (17.7)	44 (17.4)	89 (17.6)
CABG/Coronary angioplasty	27 (10.6)	24 (9.5)	51 (10.1)
Prior cerebral infarct(s)	74 (29.1)	46 (18.2)	120 (23.7)
Prior TIA(s)	39 (15.4)	28 (11.1)	67 (13.2)
Prior intracerebral hemorrhage(s)	9 (3.5)	8 (3.2)	17 (3.4)
Prior stroke, infarct vs. hemorrhage unknown	23 (9.1)	18 (7.1)	41 (8.1)
Renal disease	37 (14.6)	34 (13.4)	71 (14.0)
Dialysis	1 (0.4)	0	1 (0.2)
Existing hemiplegia or paraplegia	8 (3.1)	8 (3.2)	16 (3.2)
Clinical Characteristics & Workflow			
Type of Stroke [n (%)]			
Hemorrhagic (ICH)	0	0	0
Ischemic	52 (20.5)	45 (17.8)	97 (19.1)
TIA	1 (0.4)	0	1 (0.2)
Stroke Mimic/Undetermined	0	0	0
Stroke Severity (Median [IQR])			
Baseline LAMS	5 [3, 5]	5 [5, 5]	5 [4, 5]
ED Arrival NIHSS	17 [11, 18]	19 [18, 20]	17 [13, 19]
Reperfusion Received [n (%)]			
Intravenous thrombolysis (IVT)	39 (15.4)	37 (14.6)	76 (15.0)
Endovascular Thrombectomy (EVT)	53 (100)	45 (100)	98 (100)
IVT and/or EVT (Any Reperfusion)	53 (100)	45 (100)	98 (100)
Workflows (Median [IQR])			
Last seen normal to dosing (minutes)	70 [53, 139]	69 [61, 145]	70 [55, 139]
Dosing to IVT*	55 [42, 70]	56 [43, 67]	55 [42, 68]
Dosing to EVT**	104 [73, 113]	76 [61, 98]	87 [68, 107]

*nerinetide n=39; placebo n =37; ** nerinetide n=53; placebo n = 45

Table S10: Participant Demographics of ICH Population in FRONTIER

	Nerinetide (N = 38)	**Placebo (N = 55)**	**Total (N =93)**
Age (years; median [IQR])	68 [59, 77]	75 [67, 81]	73 [63, 80]
Sex [n (%)]			
Men	19 (50.0)	32 (58.2)	51 (54.8)
Women	19 (50.0)	23 (41.8)	42 (45.2)
Race [n (%)]			
White	14 (36.8)	35 (63.6)	49 (52.7)
Black or African American	0	1 (1.8)	1 (1.1)
Asian	22 (57.9)	19 (34.5)	41 (44.1)
Other	2 (5.2)	0	2 (2.2)
Medical History [n (%)]			
Cardiovascular	16 (42.1)	24 (43.6)	40 (43)
Congestive heart failure or pulmonary edema	1 (2.6)	3 (5.5)	4 (4.3)
Peripheral vascular disease	1 (2.6)	3 (5.5)	4 (4.3)
Vascular Risk Factors			
Hypertension	26 (68.4)	44 (80.0)	70 (75.3)
Diabetes mellitus	8 (21.1)	11 (20.0)	19 (20.4)
Hyperlipidemia	15 (39.5)	22 (40.0)	37 (39.8)
Atrial fibrillation	5 (13.2)	10 (18.2)	15 (16.1)
Tobacco (current or within past year)	6 (15.8)	12 (21.8)	18 (19.4)
CABG/Coronary angioplasty	1 (2.6)	2 (3.6)	3 (3.2)
Prior cerebral infarct(s)	10 (26.3)	3 (5.5)	13 (14)
Prior TIA(s)	2 (5.3)	4 (7.3)	6 (6.5)
Prior intracerebral hemorrhage(s)	4 (10.5)	2 (3.6)	6 (6.5)
Prior stroke, infarct vs. hemorrhage unknown	3 (7.9)	1 (1.8)	4 (4.3)
Renal disease	4 (10.5)	5 (9.1)	9 (9.7)
Dialysis	0	0	0
Existing hemiplegia or paraplegia	2 (5.3)	1 (1.8)	3 (3.2)
Clinical Characteristics & Workflow			
Type of Stroke [n (%)]			
Hemorrhagic (ICH)	38 (100)	55 (100)	93 (100)
Ischemic	0	0	0
TIA	0	0	0
Stroke Mimic/Undetermined	0	0	0
Stroke Severity (Median [IQR])			
Baseline LAMS	5 [4, 5]	5 [4, 5]	5 [4, 5]
ED Arrival NIHSS	19 [11, 22]	18 [10, 22]	18 [10, 22]
Reperfusion Received [n (%)]			
Intravenous thrombolysis (IVT)	NA	NA	NA
Endovascular Thrombectomy (EVT)	NA	NA	NA
IVT and/or EVT (Any Reperfusion)	NA	NA	NA
Workflows (Median [IQR])			
Last seen normal to dosing (minutes)	60 [44, 84]	62 [46, 87]	61 [45, 87]
Dosing to IVT*	NA	NA	NA
Dosing to EVT**	NA	NA	NA

NA= Not Applicable

Table S11: Study Drug Exposure and Compliance (mITT population)

	Nerinetide (N = 254) [n (%)]	**Placebo (N = 253) [n (%)]**	**Total (N = 507) [n (%)]**
Number of participants receiving any study drug	254 (100)	253 (100)	507 (100)
Participants who received incomplete dose by volume (< 75%)	2 (0.8)	4 (1.6)	6 (1.2)
Duration of Infusion (minutes)			
< 9 minutes	5 (2)	8 (3.2)	13 (2.6)
9 – 11 minutes (compliant)	228 (89.8)	226 (89.3)	454 (89.5)
> 11 minutes	21 (8.3)	19 (7.5)	40 (7.9)

Table S12: Relationship Between D90 mRS and Stroke Severity Measurement by LAMS or NIHSS on ED Arrival

	Correlation Coefficients – relationship with D90 mRS				Coefficients of Determination – relationship with D90 mRS			
Population	**N**	**Prehospital LAMS (r)**	**N**	**ED NIHSS (r)**	**N**	**Prehospital LAMS (r)**	**N**	**ED NIHSS (r)**
mITT	465	0.292	437	0.548	465	0.085	437	0.300
AIS	306	0.237	302	0.439	306	0.056	302	0.193
Any Reperfusion	223	0.219	222	0.417	223	0.048	222	0.174
Thrombolytics	204	0.232	203	0.444	204	0.054	203	0.197
EVT	91	0.118	91	0.138	91	0.014	91	0.019
AIS without reperfusion	85	0.339	82	0.701	85	0.115	82	0.492
ICH	86	0.263	74	0.722	86	0.069	74	0.522
Per Protocol	420	0.286	394	0.536	420	0.082	394	0.287

Comparison of the relationship between day 90 mRS and prehospital LAMS vs. relationship between day 90 mRS and ED Arrival NIHSS, in the various trial populations. Shown are the correlation coefficients (r) and the coefficient of determination (r-square) for patients with known day 90 mRS outcomes (i.e., without imputation). In general, prehospital LAMS correlates poorly with day 90 outcome as compared with ED Arrival NIHSS, especially in patients with AIS and its sub-populations.

In the FRONTIER study, the LAMS was a poor predictor of stroke outcome in patients with ischemic stroke. Whereas the original analysis plan used the LAMS as an adjustment variable for the efficacy analysis, we found that LAMS was inferior to the baseline NIHSS in accounting for the variation in day 90. For this reason, the NIHSS was substituted for the LAMS in the exploratory analyses.

As shown in Table S12 the prehospital LAMS score correlated weakly with Day 90 outcomes as gauged using the mRS, accounting little for the variation in clinical outcomes. This correlation was weakest in patients with AIS and the various AIS sub-populations.

Additionally, in FRONTIER the LAMS was a prognostic covariate for predicting day 90 mRS. In the per-SAP analysis, the primary outcome of the study was the percentage of responders, using a sliding dichotomy on the mRS scale at Day 90. The per-SAP analysis was a regression, adjusted for the covariates of age, EMS hub and LAMS. As shown in Table S13, among the sub-populations of the participants, the LAMS variable was statistically significant only in patients with ICH, and not in any of the AIS populations. By contrast, the NIHSS on ED arrival was a highly significant baseline prognostic variable in predicting outcome in all populations.

Table S13: Comparison of the Statistical Significance of LAMS vs. NIHSS as prognostic covariates for Day 90 mRS

	Significance of Prehospital LAMS in predicting Day 90 responder per-SAP Analysis		**Significance of ED NIHSS in predicting Day 90 responder Exploratory Analysis**	
Population	**N**	**Prehospital LAMS P-value**	**N**	**ED NIHSS P-value**
mITT	506	0.027	476	< 0.001
AIS	320	0.372	316	< 0.001
Any Reperfusion	233	0.367	232	< 0.001
Thrombolytics	212	0.308	211	< 0.001
EVT	98	0.985	98	0.049
AIS without reperfusion	89	0.393	86	< 0.001
ICH	93	0.003	81	< 0.001
PP	459	0.043	431	< 0.001

The analysis for the LAMS was the analysis of the primary outcome (responder analysis) adjusted for Age, EMS hub and LAMS score as per SAP. The analysis for ED Arrival NIHSS was the same, except that the ED Arrival NIHSS was swapped for the LAMS score variable. Shown are the p-values for the indicated stroke severity variable (LAMS vs EPA NIHSS) from the regression analysis. Among the various sub-populations of the mITT and Per-Protocol (PP) populations, the ED Arrival NIHSS significantly predicted improved outcome in all, whereas LAMS was a significant predictor covariate only in patients with ICH but not in the AIS sub-populations.

Table S14: Imputed Efficacy Analysis, Adjusted for Age + Baseline NIHSS (Populations of Interest)

Outcome Measure	N	aOR (95% CI)	aRR (95% CI)	p-value	N	aOR (95% CI)	aRR (95% CI)	p-value
	mITT				**AIS**			
mRS Responder	476	1.17 (0.79-1.73)	1.08 (0.89-1.31)	0.436	316	1.47 (0.91-2.38)	1.20 (0.96-1.50)	0.110
mRS Shift (cOR [95% CI])	476	1.26 (0.91-1.75)		0.164	316	1.63 (1.08-2.44)		0.019
Worsening of Stroke	476	0.54 (0.29-1.02)	0.65 (0.41- 1.02)	0.056	316	0.48 (0.23-1.03)	0.56 (0.31- 1.02)	0.059
Mortality	476	0.91 (0.56-1.49)	0.94 (0.68- 1.30)	0.703	316	0.67 (0.34-1.31)	0.76 (0.48- 1.20)	0.243
mRS 0-1	476	1.3 (0.87-1.96)	1.16 (0.92- 1.47)	0.199	316	1.77 (1.07-2.91)	1.38 (1.04-1.84)	0.022
Barthel ≥ 95	476	1.29 (0.85-1.95)	1.09 (0.95- 1.27)	0.229	316	1.31 (0.8-2.14)	1.11 (0.92- 1.34)	0.273
	Any Reperfusion				**Thrombolytics**			
mRS Responder	232	1.62 (0.93-2.83)	1.24 (0.96- 1.59)	0.086	211	1.84 (1.01-3.36)	1.31 (1.00- 1.70)	0.041
mRS Shift (cOR [95% CI])	232	1.85 (1.15-2.99)		0.011	211	2.18 (1.31-3.62)		0.003
Worsening of Stroke	232	0.38 (0.16-0.92)	0.45 (0.22- 0.93)	0.032	211	0.32 (0.12-0.82)	0.38 (0.17- 0.84)	0.017
Mortality	232	0.68 (0.32-1.44)	0.76 (0.45-1.29)	0.314	211	0.45 (0.19-1.08)	0.59 (0.33- 1.04)	0.072
mRS 0-1	232	1.99 (1.11-3.54)	1.46 (1.06- 2.01)	0.016	211	2.10 (1.14-3.88)	1.50 (1.07- 2.11)	0.014
Barthel ≥ 95	232	1.49 (0.84-2.63)	1.16 (0.93- 1.44)	0.167	211	1.65 (0.9-3.04)	1.20 (0.96-1.51)	0.103
	EVT				**AIS without Reperfusion**			
mRS Responder	98	1.49 (0.64-3.48)	1.20 (0.81-1.77)	0.351	86	1.20 (0.4-3.58)	1.07 (0.70- 1.66)	0.748
mRS Shift (cOR [95% CI])	98	1.78 (0.84-3.77)		0.134	86	0.86 (0.39-1.93)		0.721
Worsening of Stroke	98	0.76 (0.22-2.64)	0.79 (0.27- 2.33)	0.666	86	1.01 (0.16-6.28)	1.02 (0.40, 2.52)	0.986
Mortality	98	1.12 (0.39-3.17)	1.08 (0.52- 2.23)	0.834	86	0.57 (0.11-2.79)	0.73 (0.31- 1.71)	0.476
mRS 0-1	98	2.07 (0.85-5.02)	1.54 (0.91- 2.60)	0.095	86	1.20 (0.38-3.83)	1.09 (0.64-1.83)	0.757
Barthel ≥ 95	98	1.23 (0.51-2.95)	1.07 (0.79- 1.46)	0.644	86	0.98 (0.33-2.89)	0.99 (0.71- 1.39)	0.971
	ICH				**PP**			
mRS Responder	81	0.94 (0.2-4.35)	0.97 (0.47- 2.00)	0.941	431	1.06 (0.7-1.59)	1.03 (0.84- 1.25)	0.796
mRS Shift (cOR [95% CI])	81	0.54 (0.2-1.45)		0.219	431	1.22 (0.87-1.72)		0.255
Worsening of Stroke	81	1.69 (0.45-6.40)	1.28 (0.69- 2.37)	0.438	431	0.54 (0.28-1.05)	0.63 (0.39- 1.04)	0.067
Mortality	81	2.05 (0.60-6.98)	1.30 (0.85- 2.01)	0.251	431	0.93 (0.56-1.56)	0.95 (0.67- 1.35)	0.795
mRS 0-1	81	0.22 (0.02-2.09)	0.43 (0.12- 1.55)	0.126	431	1.25 (0.81-1.91)	1.14 (0.89- 1.46)	0.307
Barthel ≥ 95	81	2.41 (0.59-9.87)	1.49 (0.80- 2.80)	0.221	431	1.25 (0.81-1.92)	1.08 (0.93- 1.26)	0.311

mITT= modified intent to treat; AIS = Acute Ischemic Stroke; Any Reperfusion = population receiving thrombolysis or mechanical thrombectomy or both; Thrombolytics = received intravenous thrombolysis; EVT = received mechanical thrombectomy; AIS without reperfusion = AIS diagnosis not treated with any reperfusion therapy; ICH = hemorrhagic stroke; PP = per protocol. Binary logistic regression was used to derive odds ratios (aOR) and risk ratios (aRR). These were adjusted for age and baseline NIHSS in the ED and are presented with their 95% confidence intervals. mRS shift analysis was conducted using an ordinal logistic regression, and is reported as a common odds ratio (cOR) for improved functional outcome, adjusted for aged and NIHSS in the ED.

Table S15: Unadjusted Efficacy Analysis

Outcome Measure	Total	Nerinetide [n/N (%)]	Placebo [n/N (%)]	Absolute effect size in favour of nerinetide	OR (95% CI)	RR (95% CI)
mITT						
mRS Responder	466	108/229 (47.2)	104/237 (43.9)	3.30%	1.14 (0 .79-1.64)	1.07 (0.88- 1.31)
Worsening of Stroke	507	28/254 (11)	38/253 (15)	4.00%	0.7 (0.42-1.18)	0.73 (0.47-1.16)
Mortality	475	39/235 (16.6)	45/240 (18.8)	2.20%	0.86 (0.54-1.38)	0.89 (0.60-1.31)
mRS 0-1	466	87/229 (38)	80/237 (33.8)	4.20%	1.2 (0.82-1.76)	1.13 (0.88-1.44)
Barthel ≥ 95	461	115/225 (51.1)	117/236 (49.6)	1.50%	1.06 (0.74-1.53)	1.03 (0.86-1.24)
AIS						
mRS Responder	307	83/166 (50)	63/141 (44.7)	5.30%	1.24 (0.79-1.94)	1.12 (0.88-1.42)
Worsening of Stroke	321	17/172 (9.9)	21/149 (14.1)	4.20%	0.67 (0.34-1.31)	0.7 (0.38-1.28)
Mortality	309	23/166 (13.9)	23/143 (16.1)	2.20.%	0.84 (0.45-1.56)	0.86 (0.51-1.47)
mRS 0-1	307	68/166 (41)	45/141 (31.9)	9.00%	1.48 (0.93-2.37)	1.28 (0.95-1.74)
Barthel ≥ 95	302	89/162 (54.9)	72/140 (51.4)	3.50%	1.15 (0.73-1.81)	1.07 (0.86-1.32)
Any Reperfusion						
mRS Responder	223	69/123 (56.1)	47/100 (47)	9.10%	1.44 (0.85-2.44)	1.19 (0.92-1.55)
Worsening of Stroke	233	10/129 (7.8)	16/104 (15.4)	7.60%	0.46 (0.20-1.05)	0.5 (0.24-1.06)
Mortality	223	15/123 (12.2)	17/100 (17)	4.80%	0.68 (0.32-1.42)	0.72 (0.38-1.36)
mRS 0-1	223	57/123 (46.3)	33/100 (33)	13.30%	1.75 (1.02-3.02)	1.4 (1.00-1.97)
Barthel ≥ 95	222	72/122 (59)	53/100 (53)	6.00%	1.28 (0.75-2.17)	1.11 (0.88-1.41)
Thrombolytics						
mRS Responder	204	62/112 (55.4)	42/92 (45.7)	9.70%	1.48 (0.85- 2.56)	1.21 (0.92-1.60)
Worsening of Stroke	212	8/116 (6.9)	15/96 (15.6)	8.70%	0.4 (0.17-0.97)	0.44 (0.20-0.99)
Mortality	204	12/112 (10.7)	16/92 (17.4)	6.70%	0.57 (0.26- 1.26)	0.62 (0.31-1.24)
mRS 0-1	204	51/112 (45.5)	30/92 (32.6)	12.90%	1.73 (0.98-3.06)	1.4 (0.98-2.00)
Barthel ≥ 95	203	66/111 (59.5)	47/92 (51.1)	8.40%	1.4 (0.81-2.45)	1.16 (0.90-1.50)
EVT						
mRS Responder	91	28/49 (57.1)	22/42 (52.4)	4.80%	1.21 (0.53- 2.76)	1.09 (0.75-1.59)
Worsening of Stroke	98	6/53 (11.3)	6/45 (13.3)	2.00%	0.83 (0.26-2.65)	0.85 (0.29-2.45)
Mortality	91	9/49 (18.4)	7/42 (16.7)	-1.70%	1.12 (0.39-3.23)	1.1 (0.45-2.70)
mRS 0-1	91	23/49 (46.9)	14/42 (33.3)	13.60%	1.77 (0.76-4.12)	1.41 (0.84-2.37)
Barthel ≥ 95	91	29/49 (59.2)	26/42 (61.9)	-2.70%	0.89 (0.39-2.06)	0.96 (0.69-1.33)
AIS without Reperfusion						
mRS Responder	85	15/43 (34.9)	17/42 (40.5)	-5.60%	0.76 (0.32-1.81)	0.84 (0.49-1.46)
Worsening of Stroke	89	7/43 (16.3)	5/46 (10.9)	-5.40%	1.55 (0.47-5.05)	1.46 (0.50-4.27)
Mortality	87	7/43 (16.3)	6/44 (13.6)	-2.60%	1.41 (0.46-4.29)	1.33 (0.50-3.53)
mRS 0-1	85	12/43 (27.9)	13/42 (31)	-3.00%	0.84 (0.33-2.10)	0.88 (0.45-1.71)
Barthel ≥ 95	81	18/40 (45)	20/41 (48.8)	-3.80%	0.57 (0.25-1.30)	0.76 (0.50-1.15)
ICH						
mRS Responder	86	8/34 (23.5)	9/52 (17.3)	6.20%	1.47 (0.52-4.18)	1.36 (0.58-3.18)
Worsening of Stroke	93	11/38 (28.9)	17/55 (30.9)	2.00%	0.91 (0.37-2.23)	0.94 (0.50-1.77)
Mortality	88	14/35 (40)	22/53 (41.5)	1.50%	0.94 (0.40-2.22)	0.96 (0.57-1.62)
mRS 0-1	86	2/34 (5.9)	6/52 (11.5)	-5.70%	0.48 (0.00-2.24)	0.51 (0.11-2.38)
Barthel ≥ 95	86	9/34 (26.5)	7/52 (13.5)	13.00%	2.31 (0.79-6.76)	1.97 (0.81-4.78)
PP						
mRS Responder	421	96/208 (46.2)	97/213 (45.5)	0.60%	1.03 ([0.70-1.50)	1.01 (0.82-1.25)
Worsening of Stroke	460	24/231 (10.4)	34/229 (14.8)	4.50%	0.66 (0.38-1.16)	0.7 (0.43-1.14)
Mortality	429	35/213 (16.4)	40/216 (18.5)	2.10%	0.87 (0.53-1.42)	0.89 (0.59-1.34)
mRS 0-1	421	77/208 (37)	73/213 (34.3)	2.70%	1.13 (0.76-1.68)	1.08 (0.84-1.40)
Barthel ≥ 95	419	105/206 (51)	106/213 (49.8)	1.20%	1.05 (0.72-1.54)	1.02 (0.85-1.24)

mITT= modified intent to treat; AIS = Acute Ischemic Stroke; Any Reperfusion = population receiving thrombolysis or mechanical thrombectomy or both; Thrombolytics = received intravenous thrombolysis; EVT = received mechanical thrombectomy; AIS without reperfusion = AIS diagnosis not treated with any reperfusion therapy; ICH = hemorrhagic stroke; PP = per protocol. Odds ratios (OR) and risk ratios (RR) were unadjusted and are presented with their 95% confidence intervals.

Table S16: Summary of Treatment Emergent Adverse Events (Safety Population)

Preferred Term [n (%))	Nerinetide (N = 265)	Placebo (N = 267)	Total (N = 532)
Headache	64 (24.2)	66 (24.7)	130 (24.4)
Hypertension	55 (20.8)	58 (21.7)	113 (21.2)
Urinary incontinence	48 (18.1)	50 (18.7)	98 (18.4)
Constipation	44 (16.6)	36 (13.5)	80 (15.0)
Vomiting	35 (13.2)	45 (16.9)	80 (15.0)
Urinary tract infection	39 (14.7)	39 (14.6)	78 (14.7)
Anal incontinence	37 (14.0)	39 (14.6)	76 (14.3)
Stroke in evolution	31 (11.7)	41 (15.4)	72 (13.5)
Nausea	37 (14.0)	32 (12.0)	69 (13.0)
Contusion	33 (12.5)	26 (9.7)	59 (11.1)
Hypokalaemia	29 (10.9)	30 (11.2)	59 (11.1)
Oedema peripheral	29 (10.9)	23 (8.6)	52 (9.8)
Hypotension	33 (12.5)	18 (6.7)	51 (9.6)
Urinary retention	25 (9.4)	24 (9.0)	49 (9.2)
Arthralgia	26 (9.8)	22 (8.2)	48 (9.0)
Atrial fibrillation	25 (9.4)	23 (8.6)	48 (9.0)
Agitation	22 (8.3)	18 (6.7)	40 (7.5)
Bradycardia	19 (7.2)	19 (7.1)	38 (7.1)
Pyrexia	18 (6.8)	19 (7.1)	37 (7.0)
Restlessness	19 (7.2)	18 (6.7)	37 (7.0)
Pain in extremity	20 (7.5)	13 (4.9)	33 (6.2)
Haematuria	18 (6.8)	14 (5.2)	32 (6.0)
Troponin increased	15 (5.7)	16 (6.0)	31 (5.8)
Cough	16 (6.0)	14 (5.2)	30 (5.6)
Hypoventilation	15 (5.7)	14 (5.2)	29 (5.5)
Fall	11 (4.2)	17 (6.4)	28 (5.3)
Anaemia	15 (5.7)	12 (4.5)	27 (5.1)
Haemorrhagic transformation stroke	14 (5.3)	12 (4.5)	26 (4.9)
Pain	14 (5.3)	11 (4.1)	25 (4.7)
Back pain	15 (5.7)	9 (3.4)	24 (4.5)
Confusional state	15 (5.7)	9 (3.4)	24 (4.5)
Insomnia	9 (3.4)	15 (5.6)	24 (4.5)
Diarrhoea	8 (3.0)	15 (5.6)	23 (4.3)
Tachycardia	14 (5.3)	9 (3.4)	23 (4.3)
Delirium	14 (5.3)	8 (3.0)	22 (4.1)
Dyslipidaemia	16 (6.0)	6 (2.2)	22 (4.1)
Haematoma	14 (5.3)	7 (2.6)	21 (3.9)

Table S17: Summary of Treatment Emergent Adverse Events Resulting in Death (Safety Population)

System Organ Class Preferred Term	Nerinetide (N = 265) [n (%)]	Placebo (N = 267) [n (%)]	Total (N = 532) [n (%)]
Number of TEAE resulting in death	39	43	82
Participants with at least one TEAE resulting in death	39 (14.7)	43 (16.1)	82 (15.4)
Nervous system disorders	28 (10.6)	35 (13.1)	63 (11.8)
Stroke in evolution	21 (7.9)	29 (10.9)	50 (9.4)
Haemorrhagic transformation stroke	4 (1.5)	2 (0.7)	6 (1.1)
Haemorrhage intracranial	0	2 (0.7)	2 (0.4)
Haemorrhagic stroke	1 (0.4)	1 (0.4)	2 (0.4)
Ischaemic stroke	1 (0.4)	1 (0.4)	2 (0.4)
Neurological decompensation	1 (0.4)	0	1 (0.2)
Respiratory, thoracic and mediastinal disorders	5 (1.9)	5 (1.9)	10 (1.9)
Aspiration	3 (1.1)	2 (0.7)	5 (0.9)
Respiratory failure	1 (0.4)	2 (0.7)	3 (0.6)
Pneumonia aspiration	0	1 (0.4)	1 (0.2)
Pulmonary embolism	1 (0.4)	0	1 (0.2)
General disorders and administration site conditions	1 (0.4)	1 (0.4)	2 (0.4)
Death	1 (0.4)	0	1 (0.2)
Multiple organ dysfunction syndrome	0	1 (0.4)	1 (0.2)
Infections and infestations	1 (0.4)	1 (0.4)	2 (0.4)
Sepsis	1 (0.4)	1 (0.4)	2 (0.4)
Metabolism and nutrition disorders	1 (0.4)	1 (0.4)	2 (0.4)
Failure to thrive	1 (0.4)	1 (0.4)	2 (0.4)
Cardiac disorders	1 (0.4)	0	1 (0.2)
Ischaemic cardiomyopathy	1 (0.4)	0	1 (0.2)
Injury, poisoning and procedural complications	1 (0.4)	0	1 (0.2)
Subdural haematoma	1 (0.4)	0	1 (0.2)
Neoplasms benign, malignant and unspecified	1 (0.4)	0	1 (0.2)
Pancreatic carcinoma metastatic	1 (0.4)	0	1 (0.2)

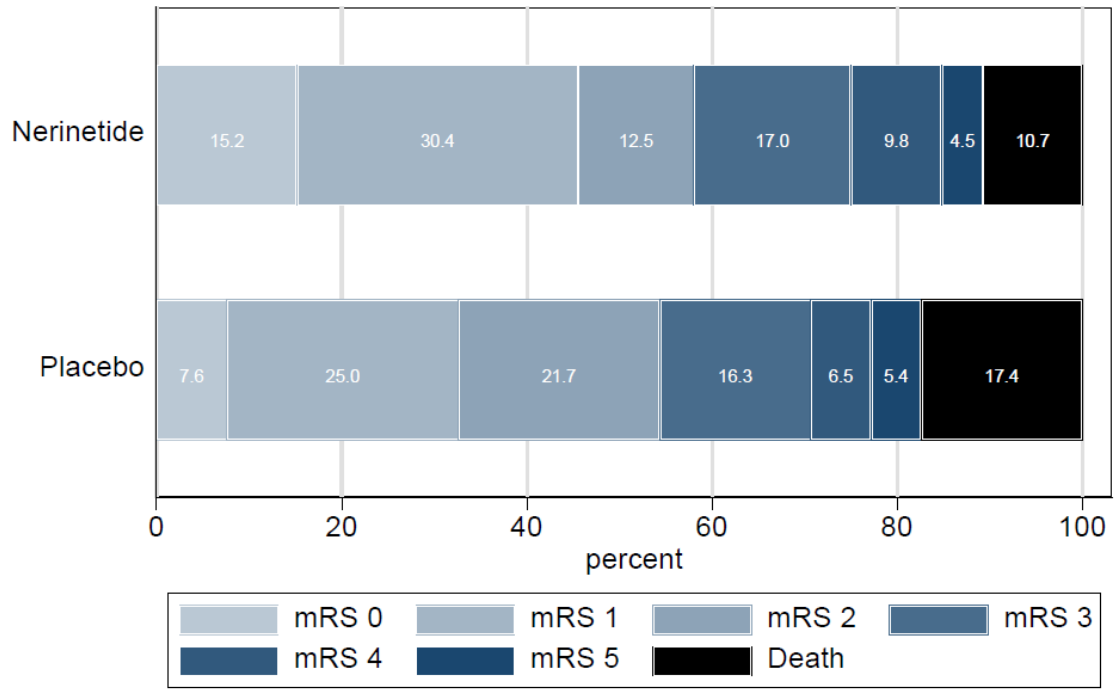
Table S18: Trial Inclusion and Exclusion Criteria

Inclusion Criteria 1) Provisional diagnosis of acute stroke and subject was a candidate for a redirect and transport to a designated stroke center following the local approved acute stroke triage tool 2) Aged 40-95 years, inclusive 3) Respiratory rate 12-24 breaths per minute 4) Oxygen saturation ≥ 90% on room air 5) Systolic blood pressure 90-220 mmHg 6) Known or estimated weight 45-120 kg 7) Last seen in a usual state of health less than three hours before anticipated study drug initiation 8) Independently ambulatory with or without devices prior to event. 9) Los Angeles Motor Scale (LAMS) score of 2-5 for ≥ 15 minutes and LAMS score remains 2-5 at time of randomization
Exclusion Criteria 1) Lack of IV access 2) Canadian Triage and Acuity Scale (CTAS) Level 1 and/or uncorrected airway, breathing or significant circulatory problem 3) Blood sugar < 3 mmol/L (< 55 mg/dL) 4) Seizure at onset of symptoms or observed by paramedic 5) Glasgow Coma Score (GCS) < 10 6) Major head trauma in the last three months 7) Recent stroke in the last three months 8) Known or presumptive signs of pregnancy or breastfeeding 9) Prisoner 10) Long term care facility resident 11) Known advance directive to not resuscitate 12) Known participation in a clinical trial with an investigational drug or device within 30 days preceding this trial 13) Pre-existing neurologic, psychiatric, or advanced systemic disease that would preclude obtaining the neurological or functional outcome evaluations.

Table S19: Schedule of Assessments

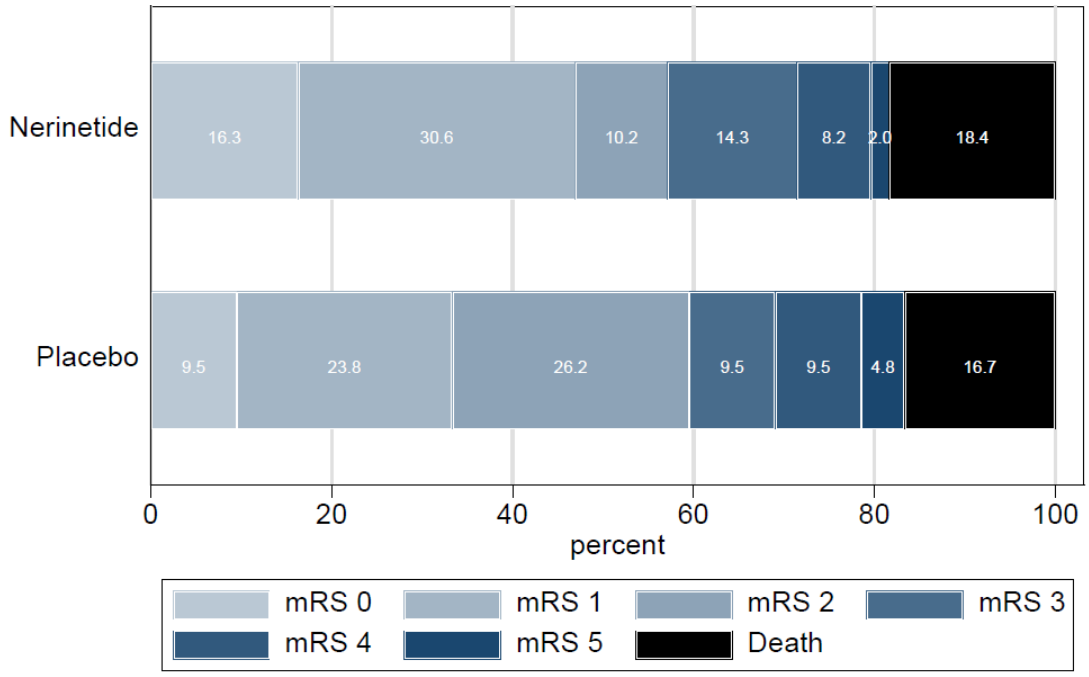
Day	**Baseline Day 1**	**Day 1**	**Day 2**	**Day 4**	**Day 30**	**Day 90**
Window	**Pre-ED Arrival**	**ED Arrival**	**18-30 hr**	**±1 day**	**±7 day**	**±30 day**
In Ambulance						
Brief Medical and Surgical History	X					
Brief Demographics	X					
Local approved stroke protocol, LAMS, GCS, Inclusion and exclusion criteria	X					
Blood Pressure, Heart Rate	X					
Weight	X					
Study Drug Infusion	X					
In Hospital						
Complete Medical and Surgical History		X	X			
Blood Pressure, Heart Rate		X	X	X		
Temperature, SaO_2		X	X			
Blood Labs		X	X			
Pregnancy Test (if applicable)		X				
Electrocardiogram		X				
Serious Adverse Events	Collected from Day 0 to Day 90/end of study					
Adverse Events	Collected from Day 0 to Day 30					
Prior and Concomitant Medications	Collected from 3 days prior to Day 4					
NIHSS		X	X	X		X
mRS			X	X	X	X
Barthel Index				X	X	X
EQ-5D-5L					X	X

Figure S1: mRS Shift at Day 90- Thrombolysis Population (N=204)



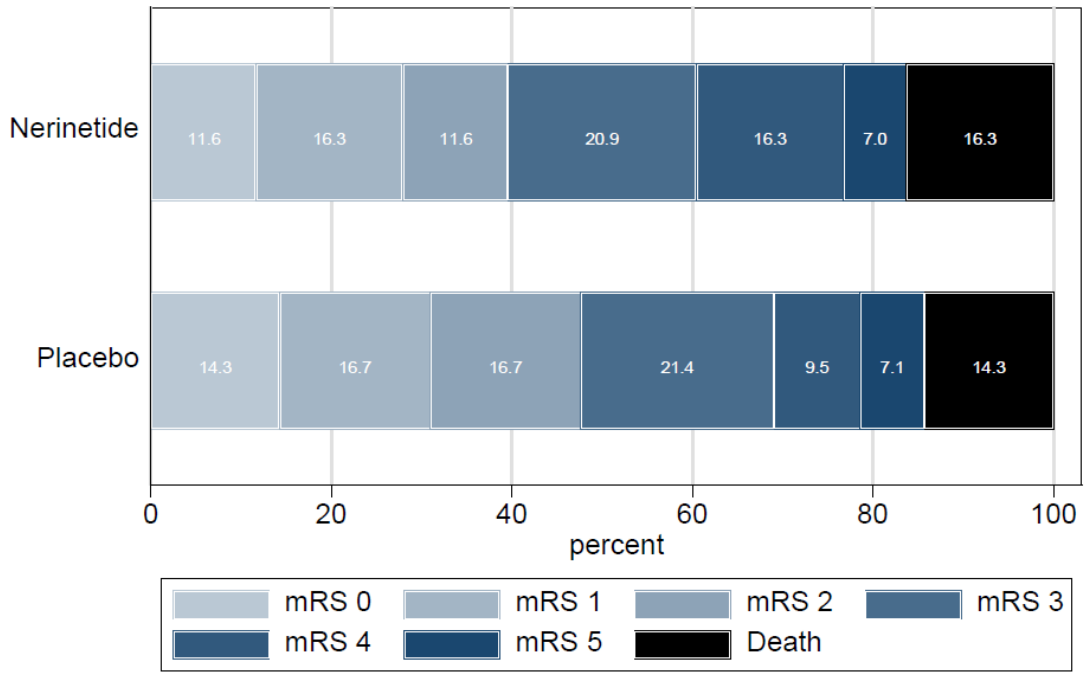
Day 90 modified Rankin Scale (mRS) distribution by nerinetide and placebo groups for the thrombolysis population. Results of the proportional odds model, adjusted for age and baseline NIHSS, are as provided in Table 3.

Figure S2: mRS Shift at Day 90- EVT Population (N=91)



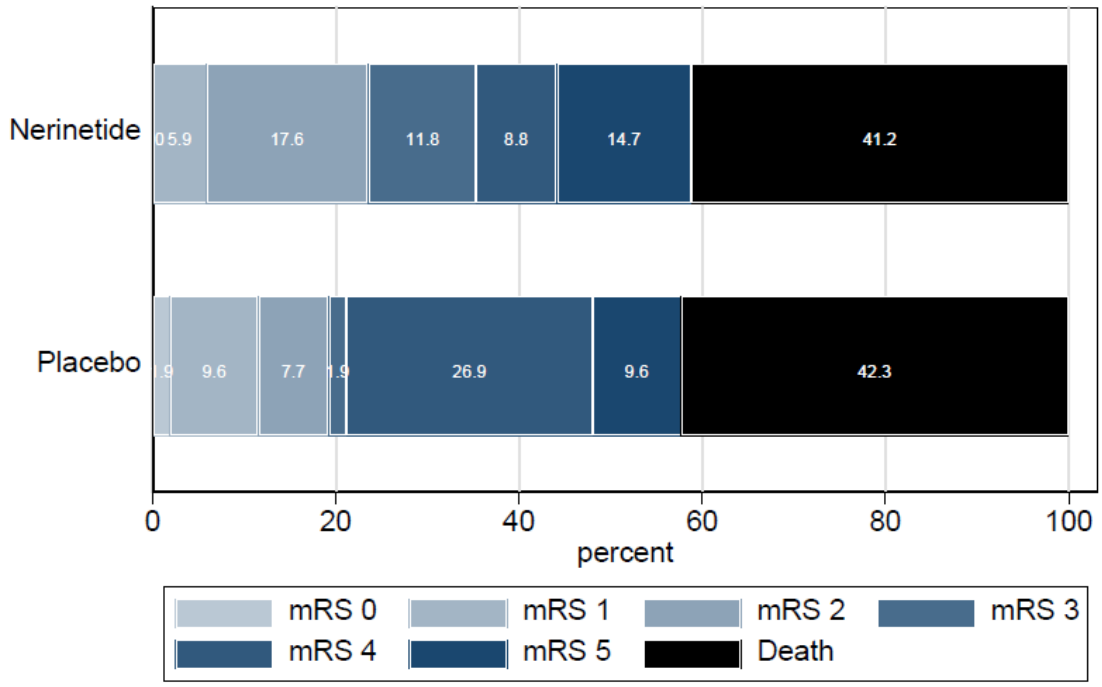
Day 90 modified Rankin Scale (mRS) distribution by nerinetide and placebo groups for the endovascular thrombectomy population. Results of the proportional odds model, adjusted for age and baseline NIHSS, are as provided in Table 3.

Figure S3: mRS Shift at Day 90- AIS without Reperfusion Population (N=85)



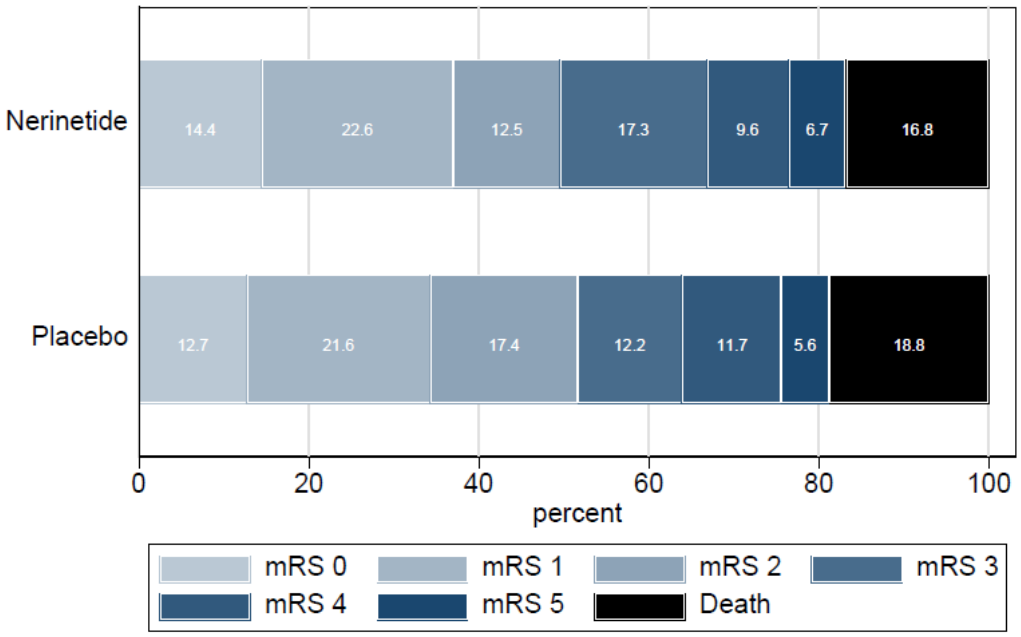
Day 90 modified Rankin Scale (mRS) distribution by nerinetide and placebo groups for the AIS without reperfusion population. Results of the proportional odds model, adjusted for age and baseline NIHSS, are as provided in Table 3.

Figure S4: mRS Shift at Day 90- ICH Population (N=86)



Day 90 modified Rankin Scale (mRS) distribution by nerinetide and placebo groups for the intracerebral hemorrhage (ICH) population. Results of the proportional odds model, adjusted for age and baseline NIHSS, are as provided in Table 3.

Figure S5: mRS Shift at Day 90- Per-Protocol Population (N=421)



Day 90 modified Rankin Scale (mRS) distribution by nerinetide and placebo groups for the per-protocol population. Results of the proportional odds model, adjusted for age and baseline NIHSS, are as provided in Table 3.

TRIAL PROTOCOL

A Multicenter, Randomized, Double-Blind, Placebo-Controlled Study to Determine the Efficacy and Safety of Intravenous NA-1 Initiated by Paramedics in the Field for Acute Cerebral Ischemia Within Three Hours of Symptom Onset (FRONTIER)

PROTOCOL NA-1-005

VERSION 8.0 (AMENDMENT 7)

DATE: 29 September 2021

SPONSOR

NoNO Inc.

479A Wellington Street West
Toronto, Ontario, Canada
M5V 1E7

File Number:

clinicaltrials.gov number **NCT02315443**

1 General information

1.1 Signatures of Approval

Protocol No. NA-1-005 Version 8.0 (Amendment No. 7)

Study Title:

A Multicenter, Randomized, Double-Blind, Placebo-Controlled Study to Determine the Efficacy and Safety of Intravenous NA-1 Initiated by Paramedics in the Field for Acute Cerebral Ischemia Within Three Hours of Symptom Onset

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 on Harmonization (ICH) Guideline for Good Clinical Practice (GCP), the Tri-Council Policy Statement (2), and all applicable regulatory requirements.

NoNO Inc.

_____ _____
Michael Tymianski, M.D., Ph.D., F.R.C.S.C. Date
President and CEO

Principal Investigator

_____ _____
Jim Christenson M.D. F.R.C.P.C. Date
Coordinating Investigator

Clinical Site: _____

Site Qualified/Principal Investigator

_____ _____
Name Date
Title

DOCUMENT HISTORY	
Document	**Date**
Version 1.0 (Original Protocol)	July 29, 2013
Version 2.0 (Amendment No 1)	January 14, 2014
Version 3.0 (Amendment No 2)	September 10, 2015
Version 4.0 (Amendment No 3)	May 2, 2016
Version 5.0 (Amendment No 4)	April 6, 2017
Version 6.0 (Amendment No 5)	March 27, 2019
Version 7.0 (Amendment No 6)	May 19, 2021

1.2 Table of Contents

1.3 List of Figures and Tables

1.4 List of Abbreviations

3PVO	3 Pial Vessel Occlusion
ACA	Anterior Cerebral Artery
AE	Adverse Event
AESI	Adverse Event of Special Interest
ABC	Airway, Breathing, Circulation
AIS	Acute Ischemic Stroke
ALDS	American Medical Center Linear Disability Score
ANCOVA	Analysis of Covariance
ANOVA	Analysis of Variance
ASA	Acetylsalicylic Acid
ASPECTS	Alberta Stroke Program Early CT Score
Ca	Calcium
CBC	Complete Blood Count
CRF	Case Report Form
CT	Computerized Tomography
CTA	Computerized Tomography Angiogram
CTAS	Canadian Triage and Acuity Scale
DWI	Diffusion Weighted Imaging
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
ED	Emergency Department
EMS	Emergency Medical Services
ENACT	Evaluating Neuroprotection in Coiling Therapy
FAST-MAG	Field Administration of Stroke Therapy - Magnesium
FDA	Food and Drug Administration
FLAIR	Fluid-Attenuating Inversion Recovery
GCP	Good Clinical Practice
GCS	Glasgow Coma Scale
GMP	Good Manufacturing Practice
ICH	International Conference on Harmonization
IDMC	Independent Data Monitoring Committee
INR	International Normalized Ratio
IV	Intravenous
LAMS	Los Angeles Motor Scale
LAR	Legally Authorized Representative
MAP	Mean Arterial Pressure
MCA	Middle Cerebral Artery
MCAO	Middle Cerebral Artery Occlusion
MedDRA	Medical Dictionary for Regulatory Activities
mITT	Modified Intent-to-Treat
mLAPSS	Modified Los Angeles Prehospital Stroke Screen
MRI	Magnetic Resonance Imaging
mRS	Modified Rankin Scale
NaCL	Sodium Chloride
NHPSS	Non-Human Primate Stroke Score

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
pH	Potential Hydrogen
pMCAO	Permanent Middle Cerebral Artery Occlusion
PSD-95	Post-synaptic Density 95
PTT	Prothrombin Time
REB	Research Ethics Board
RFA	Rankin Focused Assessment
RNA	Ribonucleic Acid
RR	Risk Ratio
SAE	Serious Adverse Event
SAH	Subarachnoid Hemorrhage
SaO_2	Oxygen Saturation
SOC	System Organ Class
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reaction
TICI	Thrombolysis in Cerebral Infarction
tMCAO	Temporary Middle Cerebral Artery Occlusion
TNK	Tenecteplase
tPA	Tissue Plasminogen Activator
TPD	Therapeutics Product Directorate
VAS	Visual Analog Scale
WFNS	World Federation of Neurological Surgeons

1.5 Study Synopsis

Title	A Multicenter, Randomized, Double-Blind, Placebo-Controlled Study to Determine the Efficacy and Safety of Intravenous NA-1 Initiated by Paramedics in the Field for Acute Cerebral Ischemia Within Three Hours of Symptom Onset
Trial Code	FRONTIER (NA-1-005)
Trial Objectives	Nerinetide (NA-1) is being developed as an emergency drug aimed at reducing global disability in patients with acute cerebral ischemia if administered within the first three hours after symptom onset. The primary objective is to determine the efficacy of nerinetide in reducing global disability in patients with acute stroke. The secondary objectives are to determine the efficacy of nerinetide in: • Reducing functional dependence. Reducing mortality rate • Reducing worsening of stroke • Improving neurological outcome. • Improving activities of daily living. The leading safety objectives are to determine the effect of administering a target dose of 2.60 mg/kg (up to a maximum dose of 270 mg) intravenous (IV) infusion of nerinetide within three hours of symptom onset by paramedics in the field on serious adverse events (SAEs) and 90-day mortality.
Trial Design	This trial is a multicenter, randomized, double-blind, placebo-controlled, single dose study initiated prehospital in the ambulance. Subjects with suspected acute stroke will be identified in the field by licensed, trained paramedics using the approved stroke protocol in use by the local EMS system, and further screened with components of the modified Los Angeles Prehospital Stroke Scale (mLAPSS) during the inclusion/exclusion phase to increase sensitivity of identifying likely stroke patients. Stroke severity will be graded by the Los Angeles Motor Scale (LAMS). Subjects will be approved for the study by an on-call trial physician by cellular phone. The paramedics will then begin study drug administration. Randomization is defined as the moment a subject receives any amount of study drug. Upon arrival at the emergency department (ED), subjects will receive standard-of-care treatment, including thrombolytic or endovascular therapy, as appropriate.
Main Subject Selection Criteria	A total of 558 subjects with suspected acute stroke, who meet the criteria used by participating EMS for a redirect and transport to a designated stroke center, are between the ages of 40-95 years, weigh between 45-120 kg, were independently ambulatory with or without devices prior to event,

	and have a neurological deficit as measured by a LAMS score of 2-5 present for ≥ 15 minutes and whose LAMS score remains 2-5 at time of randomization, will be randomized in this study within three hours of symptom onset. Subject exclusion criteria will mirror exclusion criteria for tissue plasminogen activator (tPA) use that are implementable in the field, and also include lack of IV access, a Glasgow Coma Scale (GCS) score of < 10, major head trauma or previous stroke within the last three months, or long-term care facility resident.
Inclusion/ Exclusion Criteria	**Inclusion Criteria** Subjects meeting the following criteria may be included in the study: 1. Provisional diagnosis of acute stroke and subject is a candidate for a redirect and transport to a designated stroke center following the local approved acute stroke triage tool 2. Age 40-95 years, inclusive (a criterion of the mLAPSS) 3. Respiratory rate 12-24 breaths per minute 4. Oxygen saturation ≥ 90% on room air 5. Systolic blood pressure 90-220 mmHg 6. Known or estimated weight 45-120 kg 7. Last seen in a usual state of health less than three hours before anticipated study drug initiation 8. Independently ambulatory with or without devices prior to event. 9. LAMS score of 2-5 for ≥ 15 minutes and LAMS score remains 2-5 at time of randomization **Exclusion Criteria** Subjects meeting any of the following criteria will be excluded: 1. Lack of IV access 2. Canadian Triage and Acuity Scale (CTAS) Level 1 and/or uncorrected airway, breathing or significant circulatory problem 3. Blood sugar < 3 mmol/L (< 55 mg/dL) 4. Seizure at onset of symptoms or observed by paramedic 5. Glasgow Coma Score (GCS) < 10 6. Major head trauma in the last three months 7. Recent stroke in the last three months 8. Known or presumptive signs of pregnancy or breastfeeding 9. Prisoner 10. Long term care facility resident

	11. Known advance directive to not resuscitate 12. Known participation in a clinical trial with an investigational drug or device within 30 days preceding this trial 13. Pre-existing neurologic, psychiatric, or advanced systemic disease that would preclude obtaining the neurological or functional outcome evaluations
Countries	Canada
Treatment	Nerinetide 2.60 mg/kg (up to a maximum dose of 270 mg, or matching placebo volume) is administered as a single 10 ± 1- minute IV infusion in the upper extremity using an ambulatory infusion pump by paramedics during ambulance transportation to the receiving hospital's ED. Treatment must be administered within three hours of stroke symptom onset.
Method of Consent	All subjects will be enrolled under an exception to consent approach, as this is a time-sensitive medical emergency setting to address the urgent medical need of patients with suspected acute stroke. This approach will be implemented at the discretion of their supervising Research Ethics Board (REB). Subjects will then be informed of the study after arrival at the ED and consent will be sought for the remaining follow-up from the subject once they regain capacity or a legally authorized representative (LAR) becomes available.
Randomization Method	Randomization is by pre-specified permuted block design, allocating nerinetide or placebo in a 1:1 ratio, and is stratified by EMS hub. Randomization codes will be generated at the manufacturer level employing a computerized random number sequence and boxed by block in ascending numerical order. EMS hubs will receive single boxes of 42 vials of study drug shipped frozen containing a whole number of pre-specified permuted blocks of concealed size (e.g., 7 permuted blocks of 6 vials arranged in a 1:1 ratio of nerinetide: placebo). In order to maintain the randomization sequence at the EMS hub, trained study personnel will assign the study drug vials to ambulances in ascending numerical order based on the clear markings on the vial and the box. Personnel will also assign all 42 vials from one box before opening the next. A single study drug vial will be assigned and tracked into the ambulance and placed in the onboard miniature refrigerator per study working practices.
Sample Size Determination	There will be an estimated 80% power to detect a 12% absolute effect difference between response rate (proportion of responders) with nerinetide and placebo, at alpha level 0.05, 2-sided with a planned sample size of 506 evaluable subjects, randomized 1:1, 253 per group. The 12% absolute response rate difference is judged to be the minimally clinically important difference to justify prehospital administration of nerinetide.

	The sample size will be inflated 10% to account for loss-to-follow-up and drop-outs (n=558, 279 per group). If loss-to-follow-up and drop-outs exceeds 10% (more than by the time that original enrollment reaches approximately 80%, the sample size may be inflated an additional 5% (n = 586, 293 per group).
Duration of Study	This study consists of one 90-day study period for each subject. Follow-up assessments will be performed upon ED arrival, at 24 Hours (in person), and Days 4 (in person), 30 (in person, or by telemedicine (preferred) or by phone (last option)), and 90 (in person or by telemedicine (preferred) or by phone (last option)).
Laboratory Tests	Blood will be collected per standard-of-care at the receiving hospital. Complete blood count (CBC), electrolytes, serum creatinine, glucose, international normalized ratio (INR) and prothrombin time (PTT) will be collected upon arrival at the ED and at 24 Hours per standard of care.
Assessment of Efficacy	The primary outcome variable is the overall proportion of subjects experiencing favourable functional outcome 90 days post-randomization. Sample size projections assume that approximately 72% of randomized subjects will have acute ischemic stroke (AIS), 24% will have intracerebral hemorrhage as their stroke subtype, 4% will have stroke-mimicking conditions, and that treatment benefit is obtained mainly in patients with acute cerebral ischemia[1]. From the results from ESCAPE-NA1 trial[2], a potentially important modifier of the effect of nerinetide is that of reperfusion of the ischemic territory. To align the FRONTIER analysis for this possibility, the primary efficacy analysis will be conducted in the Primary Efficacy Analysis Population, which will be determined using a stepwise process described in the Statistical Analysis Plan. Subjects will be grouped by randomized treatment, regardless of treatment actually received. The primary hypothesis that administration of nerinetide will result in a higher rate of responders will be tested using a generalized linear model, adjusted for EMS hub, age and LAMS score, with log link to directly estimate risk ratios (RR), consistent with 2010 recommendations to avoid overestimation of treatment effects via odds ratios[3],[4]. (This is to provide the best treatment effect estimate of the absolute difference in the primary outcome variable as responses are expected to be relatively common and a direct odds ratio may overestimate the RR, and hence the absolute treatment effect, in such a model.) Summary statistics will be presented. For continuous endpoints, the summary statistics will generally include: number of subjects with data, mean, standard deviation, median, quartiles, and range. For categorical endpoints, the summary statistics will generally include: number of

	subjects in corresponding analysis population, number and percentage of subjects in each category. Additional supportive analyses using the primary outcome variable will be performed as indicated for the sub-populations indicated provided that such additional analyses are deemed to be warranted. All tests will be conducted with two-sided level of significance alpha = 0.05. A fixed sequence multiple testing procedure will control the overall experiment-wise error rate for the trial. 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. The same rule applies for each subsequent test in the fixed sequence: All tests after the first failed test are considered exploratory. There is no planned interim analysis for futility or overwhelming efficacy.
Assessment of Safety	For the safety analysis, the frequency of SAEs, 90-day mortality, adverse events (AEs), discontinuations due to AEs, vital signs, laboratory and electrocardiogram (ECG) findings will be analyzed. An Independent Data Monitoring Committee (IDMC) will perform periodic safety reviews of the clinical data. The reviews will occur once 25, 50 and then 300 subjects have reached their Day 90 final study visit.

Table 1-1: Schedule of Assessments

Procedures	Baseline Day 1 (Pre-ED Arrival)	Day 1 ED Arrival	Day 2 (24 Hours ±6 Hours) in Person	Day 4 (±1 Day) in Person	Day 30[9] (±7 Days)	Day 90[9] (±30 Days)
In Ambulance						
Brief Medical and Surgical History	X					
Brief Demographics (age, sex, major vascular risk)	X					
Local approved stroke protocol, LAMS, inclusion and exclusion criteria, GCS	X					
Blood Pressure, Heart Rate	X[1]					
Weight	X[2]			X[3]		
Initiate Study Drug Infusion	X					
In Hospital						
Prior Medications (within 3 days)		X				
Complete Medical History		X	X			
Blood Pressure, Heart Rate		X[4]	X	X		
Temperature, SaO_2		X[4]	X			
CBC, electrolytes, creatinine, glucose, INR, PTT[5]		X	X			
Pregnancy Testing[6]		X				
12-lead ECG[5]		X				
Serious Adverse Events (including symptomatic hemorrhagic transformation and recurrent ischemic stroke)	Collected from Day 0 to Day 90/end of study					
Adverse Events	Collected from Day 0 to Day 30					
Concomitant Medications	Collected from Day 0 to Day 4					
NIHSS		X[7]	X	X		X
mRS			X[8]	X	X	X
Assess whether symptoms have resolved within 24 hours of symptom onset			X			
Barthel Index				X	X	X
EQ-5D-5L					X	X
ALDS						X

[1] Blood pressure to be recorded on eligibility determination, within 10 ±5 minutes or less prior to the start of study drug infusion, and immediately upon (but no later than 15 min after) completion of study drug infusion.
[2] Determined by paramedic by first asking the patient, secondly asking a family member or third, by paramedic estimation. This weight will be used for calculating the volume of study drug to be administered.
[3] Actual weight measured in hospital within 4 days. If actual weight cannot be measured due to, for example severe illness, determine weight by first asking the subject, second asking by asking a family member or third by estimation.
[4] Per standard-of-care. The assessment of vital signs closest to the 20- min time-point after ED arrival will be entered into the eCRF.
[5] Testing per standard-of-care, sample to be reported in CRF is the one closest to visit window.
[6] For women of child-bearing potential only; per standard-of-care.
[7] No more than 4 hours post-dose; per standard-of-care.
[8] Premorbid mRS status and mRS status at acute stroke hospital discharge.
[9] 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).

1.6 Sponsor, Investigators and Facilities

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2 BACKGROUND INFORMATION

2.1 The Prehospital Approach

A stroke occurs when there is a blockage of a blood vessel supplying the brain (ischemic), or bleeding into or around the brain (hemorrhagic). Stroke should be treated as a medical emergency because 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[5]. There are no pharmacological stroke therapies that have been shown to be effective if administered after three hours from symptom onset, with the possible exception of the thrombolytic agent tPA which might be of benefit up to 4.5 hours after symptom onset. The phrase "time is brain" emphasizes that brain tissue is rapidly and irretrievably lost as stroke progresses[6] and that early intervention is critical to improve a stroke victim's outcome.

For this reason, nerinetide is being developed as an emergency drug aimed at reducing global disability in patients with acute cerebral ischemia if administered within three hours of symptom onset. The central aim of this study is to demonstrate the efficacy and safety of 2.60 mg/kg (up to a maximum of 270 mg) of nerinetide administered to patients with suspected acute stroke by paramedics in the field within three hours of symptom onset. The reason for the prehospital approach is that early treatment with a safe and effective agent has the highest likelihood of providing patients with a clinical benefit. This rapid intervention will be optimized by having an on-call trial physician review inclusion/exclusion criteria by cellular phone to determine if the subject is eligible for the trial, obviating the need to divert paramedic attention from their other prehospital duties. The prehospital trial approach also ensures that study drug initiation will not interfere with standard-of-care of patients as of their arrival in the ED, particularly the evaluation for and institution of thrombolytic therapy.

New, safe, effective and widely applicable treatments for stroke are urgently needed. The current era poses multiple challenges, including the challenge of testing a putative therapy in a manner that does not conflict with current standard-of-care. Therefore, not only are new treatments required, but a novel research paradigm that addresses the need for early therapy while not interfering with and potentially bolstering the effectiveness of other standard-of-care therapies is also required for any agent to be approved for use.

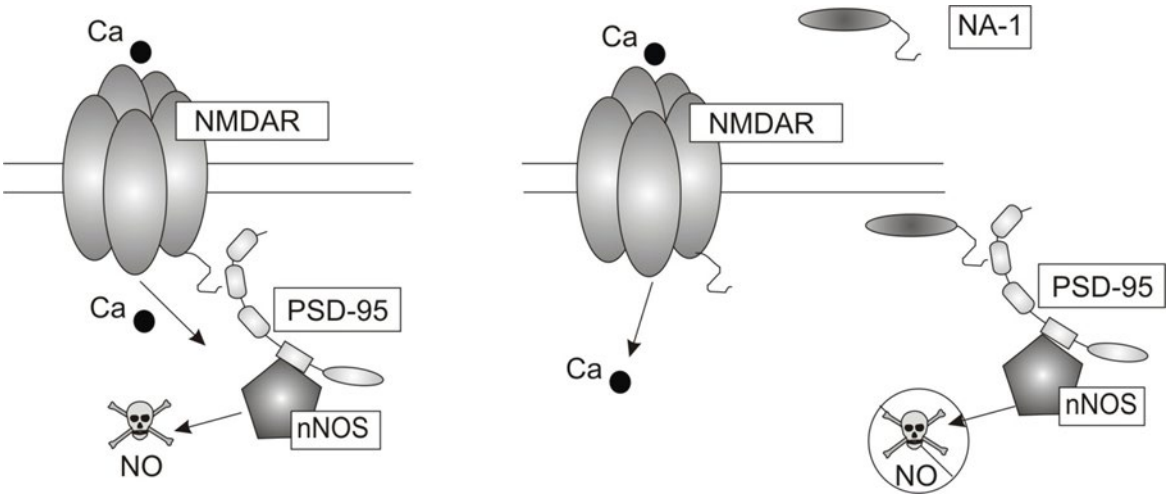
2.2 Name and Description of Investigational Product

Nerinetide (previously referred to as NA-1, these will be used interchangeably in this document) is a synthetic peptide that may provide significant benefit for the treatment of acute cerebral ischemia if administered early after symptom onset. The short window for the potential clinical efficacy of nerinetide in acute cerebral ischemia is based on the findings from multiple studies that neuroprotective therapeutic interventions for acute stroke have all been ineffective, and all had the commonality of administering the intervention in a more delayed window. This is likely due to the nature of evolution of acute stroke in the general community rather than a property of the specific intervention. Our preclinical and clinical data support this notion.

Nerinetide targets post-synaptic density 95 (PSD-95) protein, which is highly localized to the post-synaptic density in neurons of the central nervous system. Nerinetide is composed of two parts: a 9-amino acid active substance that binds to PSD-95, an intracellular component of signalling pathways regulating neuronal cell death after ischemic damage, and an 11-amino acid sequence of

the Tat protein which permits nerinetide to penetrate neuronal target cells. PSD-95, an intracellular component of signaling pathways regulating neuronal cell death after ischemic damage, functions to couple transmembrane proteins [e.g., the N-methyl-D-aspartate (NMDA) subtype of glutamate receptors] to various intracellular signaling enzymes. The nerinetide peptide was designed to inhibit the interaction between PSD-95 and the NR2B subunit of the NMDA receptor (NMDAR) to prevent or limit the onset of neuronal excitotoxicity that is associated with AIS in which glutamatergic mechanisms play a pathophysiological role (Figure 2-1). The nerinetide peptide has no known effect on the electrophysiological aspects of NMDAR channel function, but results in decreases in downstream neurotoxic signaling [e.g., the production of the toxic free radical nitric oxide (NO)] [7].

Figure 2-1: Inhibition of NO production by Nerinetide via perturbation of NMDAR-PSD-95 interactions



Ca=calcium; nNOS=neuronal nitric oxide synthase

2.3 Summary of Findings from Nonclinical Studies and Clinical Trials

Nerinetide has shown to be a promising neuroprotectant in (1) rats and in non-human primates exposed to experimental strokes when administered within three hours of stroke onset, and (2) humans experiencing procedurally-induced stroke as a consequence of endovascular repair of brain aneurysms, as measured by a reduction in the number of new lesions visualized by magnetic resonance imaging (MRI). In the human study, nerinetide was administered on average within two hours after initiation of the aneurysm repair procedure. These preclinical and clinical findings are consistent with the well-established notion that there must still be brain left to salvage in order for any neuroprotectant to result in a clinical benefit in patients with AIS[8]. Further details can be found in the current Investigator's Brochure.

2.3.1 Nonclinical Studies

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[9]. 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.

2.3.2 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 2.6 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 2.6 mg/kg is based on the safety and tolerability profile of nerinetide observed in the Phase 1 and 2 clinical trials.

In the completed ESCAPE-NA1 trial[2], 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 unadjusted reduction in mortality rate of 7.5% (relative difference of 39.6%; $p_{(nominal)}$=0.055, 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 ($p_{(nominal)} = 0.048$).

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 serious 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 was 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 was administered with alteplase there were no differences in important safety outcomes observed between the nerinetide and placebo groups.

2.3.3 Summary of Safety of Nerinetide

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.

2.4 Description of and Justification for the Route of Administration, Dosage, Dosage Regimen and Treatment Period

Nerinetide 2.60 mg/kg, up to a maximum of 270 mg (or matching placebo volume) is administered as a single 10 ± 1-minute IV infusion in the upper extremity using an ambulatory infusion pump by paramedics initiated during ambulance transportation to the receiving hospital's ED. Treatment must be administered within three hours of stroke symptom onset.

The 2.60 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 nerinetide to reduce stroke tissue damage and to improve neurological function in rats and non-human primates exposed to experimental strokes when nerinetide was administered within three hours of stroke onset, and (3) the capacity of this dose to reduce stroke tissue damage and improve neurological damage in human subjects undergoing endovascular repair of brain aneurysms in which nerinetide was administered, on average, two hours after initiation of the repair procedure. In the clinical trial, biological effectiveness of nerinetide in subjects was measured by a reduction in the number of new lesions visualized by MRI scanning post-endovascular aneurysm repair.

The current methodology for administering nerinetide in this prehospital stroke trial has been specifically designed to address the reality that stroke intervention with a neuroprotective agent must be administered as soon as possible after symptom onset if a clinical benefit is to be observed. In conventional (in-hospital) trials, once patients arrive at the treating ED, activities relating to the stroke diagnosis and establishment of the patient's candidacy for thrombolytic therapy rise to the top of the patient care paradigm. Necessarily, this relegates the initiation of any trial enrollment procedures, randomization and drug administration procedures to times that follow the typical "door-to-needle" time of the admitting hospitals. The target benchmark for such door-to-needle times is currently 60 minutes, and most hospitals achieve this target in only 30% of cases. Given that the patient, family, and physicians are consumed with thrombolytic decision-making in the first 60-75 minutes after arrival, the process of eliciting consent, performing randomization and

initiating a neuroprotective study drug will not begin until 60-75 minutes after ED arrival and typically will not be completed until 75-120 minutes after arrival[10]. As a result, a "traditional" in-hospital trial relegates the administration of the neuroprotectant study drug to a time-frame close to, or frankly beyond, the three hour limit of its potential clinical utility. In fact, of all in-hospital trials ever conducted using neuroprotectant agents, none were able to achieve a window of enrollment and initiation of therapy averaging less than four hours, a time-frame in which neuroprotection for community-acquired stroke is likely to be futile.

By contrast with the failed notion that a neuroprotectant might be beneficial within the timeframe of enrollment in previous neuroprotection trials, evidence exists that nerinetide, when administered soon after the onset of stroke (for example, in non-human primates[11], extends the time-window of benefit of reperfusion and, in animal studies of both temporary and permanent vessel occlusion, reduces infarct size[9]. Therefore, the most practical and useful time to administer a neuroprotectant, both for investigational and clinical use, is early after stroke onset. The prehospital approach meets this need.

Our approach to administering nerinetide is designed to be practical and feasible for use by paramedics in the field. It involves the paramedic using an ambulatory infusion pump to give the infusion while in consultation with an on-call trial physician for all cases. Study drug must be initiated within three hours of symptom onset. The drug infusion will begin in the field during ambulance transportation after completing the enrolment procedures. Initiation of the infusion prior to ED arrival ensures that (1) the chain of custody of the study drug remains unbroken because the patients receive a prehospital therapy by prehospital personnel and (2) the study has minimal or no impact on the evaluation of the candidacy of patients for standard-of-care stroke therapies including reperfusion therapies because randomization and initiation of the 10 ± 1 minute infusion occur prior to hospital arrival.

2.5 Study Population

A total of 558 subjects with suspected acute stroke, who meet the criteria used by participating EMS for a redirect and transport to a designated stroke center, are between the ages of 40-95 years, weigh between 45-120 kg, were independently ambulatory with or without devices prior to event, and have a neurological deficit as measured by a LAMS score of 2-5 present for ≥ 15 minutes and whose LAMS score remains 2-5 at time of randomization, will be randomized in this study within three hours of symptom onset. Subject exclusion criteria will mirror exclusion criteria for tPA use that are implementable in the field and also include lack of IV access, a GCS score of < 10, major head trauma or previous stroke within the last three months, or long-term care facility resident (see Section 5.2).

2.6 Implications of ESCAPE-NA1 Findings on FRONTIER Trial

Knowledge from prior clinical and preclinical studies has been augmented by the ESCAPE-NA1 trial, which together the rationale for the FRONTIER trial as follows:

- The neuroprotectant, nerinetide, has been demonstrated to be highly effective in reducing stroke size and improving the functional outcome of experimental animals subjected to acute stroke, including rats and primates, especially when those models involve ischemia followed by reperfusion.

- The neuroprotectant nerinetide is an anti-ischemic drug and is therefore expected to have a potential for clinical benefit only when used to treat conditions that involve cerebral ischemia. Additionally, neuroprotection is expected to slow or temporarily halt ischemic brain damage. Therefore, it is intrinsically a temporizing measure[12, 13] anticipated to be most useful in conjunction with eventual restoration of blood flow (reperfusion) to the ischemic tissue.
- ESCAPE-NA1 provided promising results in support of a neuroprotective benefit of nerinetide in AIS patients selected for endovascular reperfusion who were not previously treated with alteplase. However, the median interval from stroke onset to dosing with study drug in this trial was 201 minutes. Faster treatment intervals from stroke onset to dosing with drug may be possible in FRONTIER because dosing is conducted in the pre-hospital setting.
- ESCAPE-NA1 showed no benefit of nerinetide in participants who were previously treated with alteplase. However, in the FRONTIER trial, none of the enrolled participants would be anticipated to receive alteplase before nerinetide, as alteplase is not an agent that can currently be given in the pre-hospital setting. FRONTIER therefore affords a unique opportunity to examine the effects of nerinetide in all enrolled participants with AIS, including those who are treated with alteplase.
- Preclinical data suggests that, in the scenario in which alteplase is given after nerinetide, nerinetide treatment remains effective and may be synergistic with the effect of alteplase[14]. In FRONTIER, it is anticipated that of participants who ultimately receive alteplase, all will have received it after dosing with study drug. This study therefore provides the opportunity to explore the safety and efficacy of both agents in the scenario ischemia-reperfusion scenario in which nerinetide may be most beneficial.

FRONTIER is anticipated to enroll patients with suspected stroke who have a range of diagnoses. The principal enrolled subgroups once a diagnosis is confirmed in-hospital would be those patients with:

- Confirmed acute cerebral ischemia (AIS or TIA)
- Confirmed hemorrhagic stroke
- Stroke mimicking conditions (e.g., hemiplegic migraine, brain tumor, post-ictal state)

Additionally, among patients with acute cerebral ischemia, some may be treated with reperfusion therapies comprising mainly of:

- Alteplase after nerinetide
- EVT after nerinetide
- Alteplase + EVT after nerinetide

Results of the ESCAPE-NA1 trial provided further evidence that nerinetide is anticipated to have its greatest efficacy in patients with AIS who are subjected to a reperfusion therapy.

The knowledge gained from the separate analyses of the alteplase and no-alteplase fully-randomized strata of the ESCAPE-NA1 trial has the following important implications for FRONTIER:

1. In addition to improving functional independence, nerinetide may slow the progression of stroke, and/or reduce mortality – two additional orthogonal endpoints of clinical relevance.
2. ESCAPE-NA1 has confirmed two potentially important effect-modifying interactions that have profound implications for FRONTIER:
    A. The effectiveness of nerinetide may be reduced in the absence of adequate reperfusion.
    B. The effectiveness of nerinetide is nullified by the prior or concurrent administration of alteplase.

Incorporating knowledge learned from ESCAPE-NA1, including of the potential effect-modifying interactions of reperfusion and alteplase is key to extracting meaning from FRONTIER. This has already been done in the Phase 3 ESCAPE-NEXT trial (clinicaltrials.gov NCT04462536) which is aimed at confirming the encouraging results in the no-alteplase stratum of ESCAPE-NA1.

The potential for effect modification by lack of reperfusion will be managed in FRONTIER in planned statistical analysis. The statistical analysis plan (SAP) for the study will include all appropriate details necessary to define the Primary Efficacy Analysis Population and the various primary, secondary and tertiary efficacy analyses.

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3 TRIAL OBJECTIVES

3.1 Primary Objectives

The primary objective is to determine the efficacy of nerinetide in reducing global disability in patients with acute stroke.

3.2 Secondary Objectives

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

- Reducing functional dependence.
- Reducing mortality rate.
- Reducing worsening of stroke*
- Improving neurological outcome.
- Improving activities of daily living.

*Worsening of stroke is defined as progression, or hemorrhagic transformation, of the index stroke as documented in the study CRF that (i) is deemed life-threatening and/or (ii) results in increased disability as gauged by a ≥4 point increase from lowest NIHSS during hospitalization and/or (iii) results in death.

3.3 Tertiary Objectives

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

- Improving functional independence
- Improving health-related quality of life.
- Increasing the proportion of subjects whose symptoms fully return to baseline function within 24 hours of symptom onset.
- Increasing the proportion of subjects who receive reperfusion therapy.
- Increasing the proportion of subjects who receive thrombolysis.
- Reducing functional dependence at Day 30
- Reducing physical disability.

3.4 Leading Safety Objectives

The leading safety objectives are to determine the effect of administering a target dose of 2.60 mg/kg (up to a maximum dose of 270 mg) IV infusion of nerinetide within three hours of symptom onset by paramedics in the field on SAEs and on 90-day mortality.

4 TRIAL DESIGN

4.1 Study Endpoints

4.1.1 Primary Efficacy Endpoint

The primary efficacy endpoint is the percentage of responders, using a sliding dichotomy on the mRS scale at Day 90.

4.1.2 Secondary Efficacy Endpoints

The secondary efficacy endpoints are:

1. Shift to reduced functional dependence analyzed across the whole distribution of scores on the mRS at Day 90 or the last rating.
2. A reduction in mortality as defined by event rate (proportion, expressed as a percentage) for mortality over the 90-day study period
3. Proportion of participants with worsening of stroke* over the 90-day study period.
4. Proportion of subjects with good neurological outcome, as defined by a score of 0-1 on the NIHSS at Day 90 or the last rating.
5. Proportion of subjects with functional independence in activities of daily living, as defined by a score of ≥ 95 on the Barthel Index at Day 90 or the last rating.

*Worsening of stroke is defined as progression, or hemorrhagic transformation, of the index stroke as documented in the study CRF that (i) is deemed life-threatening and/or (ii) results in increased disability as gauged by a ≥4 point increase from lowest NIHSS during hospitalization and/or (iii) results in death.

4.1.3 Tertiary Efficacy Endpoints

The tertiary efficacy endpoints are:

1. Proportion of subjects with functional independence, as defined by a score of a) 0-2 and b) 0-1 on the mRS at Day 90 or the last rating.
2. Health-related quality of life, as measured by the EQ-5D-5L at Day 90 or the last rating.
3. Proportion of subjects with acute cerebral ischemia whose symptoms fully return to baseline function within 24 hours of symptom onset.
4. Proportion of subjects who receive any type of reperfusion therapy.
5. Proportion of subjects who receive thrombolysis.
6. Favourable outcome at Day 30 or last rating prior to Day 30, as described for the primary endpoint.
7. Physical disability, as measured by the Academic Medical Center Linear Disability Score (ALDS) at Day 90.

4.1.4 Leading Safety Endpoints

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

4.2 Description of Trial Design

This study is a multicenter, randomized, double-blind, placebo-controlled, single dose efficacy and safety study of nerinetide initiated prehospital in the ambulance. Enrolled subjects will be given a single, intravenous, target dose of 2.6 mg/kg (up to a maximum of 270 mg) of nerinetide or placebo.

A total of 558 male and female participants between the ages of 40-95 years with suspected acute stroke will be identified in the field by licensed, trained paramedics using the approved stroke protocol in use by the local EMS system. Stroke severity will be graded by the Los Angeles Motor Scale (LAMS). Subjects will be approved for the study by an on-call trial physician by cellular phone.

The paramedics will then begin study drug administration. Randomization is defined as the moment a subject receives any amount of study drug. Upon arrival at the emergency department (ED), subjects will receive standard-of-care treatment, including thrombolytic or endovascular therapy, as appropriate.

All participants 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-person visit is not possible the participant can be contacted by telemedicine (preferred) or by telephone (last option).

4.3 Randomization and Blinding

Randomization is by pre-specified permuted block design, allocating nerinetide or placebo in a 1:1 ratio, and is stratified by EMS hub. Randomization codes will be generated at the manufacturer level employing a computerized random number sequence and boxed by block in ascending numerical order. EMS hubs will receive single boxes of 42 vials of study drug shipped frozen containing a whole number of pre-specified permuted blocks of concealed size (e.g., 7 permuted blocks of 6 vials arranged in a 1:1 ratio of nerinetide: placebo). In order to maintain the randomization sequence at the EMS hub, trained study personnel will assign the study drug vials to ambulances in ascending numerical order based on the clear markings on the vial and the box. Personnel will also assign all 42 vials from one box before opening the next. A single study drug vial will be assigned and tracked into the ambulance and placed in the onboard miniature refrigerator per study working practices. Drug accountability, distribution and tracking for each EMS hub will be documented and available for monitoring purposes (see Section 4.9).

The time of randomization is defined as the moment a subject receives any amount of study drug. The moment of randomization will be rigorously documented. All patients that are randomized will be accounted for in the trial database. The paramedic will record the randomization time, which will be entered into the electronic case report form (eCRF) and ambulance call report, respectively. The paramedic will record both the vial number and time of randomization after each randomization occurs to ensure an accurate determination of all subjects randomized and exposed to the study drug. Once a subject is randomized, re-stocking participating ambulances with the next study drug vial in that EMS hub's randomization sequence will be done per the local EMS hub working practice.

All subjects, paramedics, investigators, their clinical staff, the clinical coordinating center, the data management group, independent adjudication committee, local hospital laboratories and the Sponsor staff and delegates will be blinded to the randomization codes.

The IDMC will be unblinded to safety data to ensure detailed analysis of safety. In order to ensure confidentiality and minimize bias, the safety information will be provided to the IDMC by a group that is independent of the Sponsor and blinded project team implementing the trial. A firewall will be maintained between the IDMC (unblinded) and the project staff (blinded). The IDMC will remain blinded to efficacy data throughout the trial unless significant concerns about safety develop.

The person responsible for generating the randomization codes and the study drug packaging company will be unblinded, as will the independent statistical group preparing the safety reports for the IDMC.

4.3.1 Procedure for Breaking the Randomization Code

In the event of an emergency and following a discussion with the Medical Monitor, the randomization code for an individual subject may be revealed to the site Investigator. The randomization code would then be obtained by phone or e-mail from the person responsible for generating the randomization code, following authorization by the Medical Monitor. Any case that is unblinded in this way will be documented in central files.

The unblinded person will provide only to the site Investigator by email the subject dose allocation information as well as the date of unblinding, site number, Investigator name and subject number. Any case that is unblinded in this way will be documented in central files. Only the Investigator requesting the unblinding will receive the unblinding information. It is not expected that there is any clinical instance where unblinding will be required.

Health authorities may request code-breaking in the case of an SAE as described in ICH E2A. In this case, the code will be broken only for the subject(s) in question. The information resulting from code-breaking will not be communicated to the Sponsor or Investigator. This code will be broken by the person responsible for generating the randomization code and communicated directly to the legally designated third party.

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

4.4 Trial Treatment

4.4.1 Dosage Form

The drug product, nerinetide, was manufactured under Good Manufacturing Practice (GMP) by the *Confidential Business Information* and comprises nerinetide (20 mg/mL) in 50 mM phosphate buffered saline with 0.45% NaCl, potential hydrogen (pH) 7.0, with a fill of 13.5 mL in a 30 mL syringe vial with snap-top caps for single use only.

Placebo is formulated in the same buffer used for nerinetide at the same location with slightly higher NaCl content to adjust for equivalence of osmolality between drug product and placebo. It has been confirmed that the active drug and placebo products are visually identical as clear, colorless liquids in the same container/closure system.

Nerinetide 2.60 mg/kg up to a maximum of 270 mg (or matching placebo volume) is administered as a single 10 ± 1-minute IV infusion in the upper extremity using an ambulatory infusion pump.

Each study drug vial will be individually labeled with a unique identification number by the packaging company to preserve blinding. This unique identification number will also serve as the subject's randomization number.

4.5 Study Drug and Infusion Case Packaging and Storage

Study drug vials will be shipped frozen to each depot, confirmed upon receipt, and stored in a freezer at ≤ -10°C. Temperature tag indicators will also be shipped to each central EMS hub and appropriately stored. EMS depots will track all drug and the infusion pumps. Upon removal of the study drug vial from the freezer, a temperature tag will be affixed, and the vial number will be tracked for the duration of the trial (see Section 4.7). The expiry date on the frozen vial will be amended with a new expiry label for 2 to 8°C storage will be affixed. The new expiry date (twelve months from the date of thawing) will be written on the label and the vial will be stored in the portable refrigerator/cooler of the applicable ambulance, per local working practice. Each participating ambulance will be stocked with one single use vial of nerinetide or matching placebo. A study drug vial will be accompanied by a FRONTIER case for infusion. This case will be stored at ambient temperature. The following are contained inside the case:

1) one Moog Curlin 6000 CMS™ Ambulatory Infusion Pump,
2) one pump holster,
3) one study drug administration kit (bag) containing one Moog Curlin IV Infusion Administration Set (including tubing, an integrated in-line air filter, a male luer lock, slide clamp, tubing guide and bag spike); a 20 mL syringe and blunt tip needle for transfer of the study drug from the vial to the saline IV mini-bag; three sterile wipes; and a study bracelet,
4) an infusion instruction set,
5) a weight-based dosing chart, and
6) a study chart insert.

A 50mL saline bag will be stocked on the ambulance for infusion

4.6 Accountability and Labeling

Records will be made of the receipt of study drug boxes at each EMS hub; storage; the dispensing of the individual study drug vials in ascending order; IV administration details; and study drug vial return for accountability and reconciliation purposes.

Documentation for each study drug vial will include, but may not be limited to, the following information:

1. Receipt date
2. Description of drug package, and drug product
3. Lot/Batch/other
4. Expiry and/or Manufacturing and/or retest date
5. Dispensing and return date, time and location

Drug vials will be labelled in accordance with the regulatory requirements of the Food and Drug Administration (FDA) and Health Canada's Therapeutic Products Directorate (TPD).

4.7 Study Drug Tracking

Study drug vials (nerinetide and placebo) will be packaged at the manufacturing facility and affixed with a unique vial number printed on the label. The study drug vials will be packaged in boxes of 42 and sent to each EMS hub, with instructions to assign the vials in ascending order.

Once the vials have been received at the EMS hubs, they will be inspected, and their receipt will be documented. The EMS hub will maintain drug accountability records that will include the time and date the vial was pulled from the freezer and placed either in the EMS hub refrigerator or in the ambulance refrigerator/cooler on the assigned ambulance, for monitoring purposes and tracking of expiry dates. Periodic tracking of the vials in ambulances will be performed to confirm and document the locations of the vials. Lost, expired or damaged vials or vials with a temperature excursion as indicated on the temperature tag will be reported, documented, and returned to the EMS hub.

The Curlin infusion pumps will also have a unique pump ID number for proper tracking. The pumps will be monitored by the Sponsor or designee to ensure they are operational, maintained regularly and calibrated yearly.

4.8 Disposition of Study Drug Supplies

After each randomization, the used study drug vial will e monitored prior to destruction, per the local working practice. On a routine basis, the pump's history will be downloaded to resolve any discrepancies noted in the documentation of the infusion parameters, and this information will be recorded. It will also be confirmed that sufficient battery life remains for subsequent use, and then the pump will be re-set for the next infusion. The vial will be inspected by the monitor and verified against the subject's source documents and eCRF.

Used and unused study drug vials, unused administration kits and all pumps will ultimately be returned to the Sponsor or destroyed in accordance with local institutional policies and procedures.

4.9 Study Drug Monitoring

It is acknowledged that each EMS hub has local best working practices, which will be documented for monitoring purposes. The monitor or Drug Manager will verify that:

- Study drug vials are appropriately stored in a freezer at ≤-10°C in a secure location at each EMS hub
- Study drug vials are being placed in the ambulances and stored at 2 to 8 °C in the portable refrigerator/cooler
- FRONTIER cases for infusion are available for paramedics
- Dose volume, start and stop time, and subject weight are recorded after each randomization
- Any expired, lost, damaged or out-of-specification medication has been properly reported and documented, and follow-up has been conducted
- Ambulances are being re-stocked with a new study drug vial in a timely fashion
- Pumps are being re-set

Local best working practices will be filed centrally at the EMS hub and by the Sponsor.

4.10 Case Report Forms

All source documentation will be reviewed for compliance with GCP. Case Report Forms for the study are electronic.

5 SELECTION OF SUBJECTS

5.1 Inclusion Criteria

Subjects meeting the following criteria may be included in the study:

1. Provisional diagnosis of acute stroke and subject is a candidate for a redirect and transport to a designated stroke center following the local approved acute stroke triage tool
2. Age 40-95 years, inclusive (a criterion of the Modified Los Angeles Prehospital Stroke Screen -mLAPSS)
3. Respiratory rate 12-24 breaths per minute
4. Oxygen saturation ≥ 90% on room air
5. Systolic blood pressure 90-220 mmHg
6. Known or estimated weight 45-120 kg
7. Last seen in a usual state of health less than three hours before anticipated study drug initiation
8. Independently ambulatory with or without devices prior to event.
9. Los Angeles Motor Scale (LAMS) score of 2-5 for ≥ 15 minutes and LAMS score remains 2-5 at time of randomization

5.2 Exclusion Criteria

Subjects meeting any of the following criteria will be excluded:

1. Lack of IV access
2. Canadian Triage and Acuity Scale (CTAS) Level 1 and/or uncorrected airway, breathing or significant circulatory problem
3. Blood sugar < 3 mmol/L (< 55 mg/dL)
4. Seizure at onset of symptoms or observed by paramedic
5. Glasgow Coma Score (GCS) < 10
6. Major head trauma in the last three months
7. Recent stroke in the last three months
8. Known or presumptive signs of pregnancy or breastfeeding
9. Prisoner
10. Long term care facility resident
11. Known advance directive to not resuscitate
12. Known participation in a clinical trial with an investigational drug or device within 30 days preceding this trial
13. Pre-existing neurologic, psychiatric, or advanced systemic disease that would preclude obtaining the neurological or functional outcome evaluations

6 TREATMENT OF SUBJECTS

6.1 Randomization

Subjects with suspected acute stroke will be identified in the field by licensed paramedics who have received training in basic cardiac life support, stroke recognition, and specific procedures relevant to the proposed study.

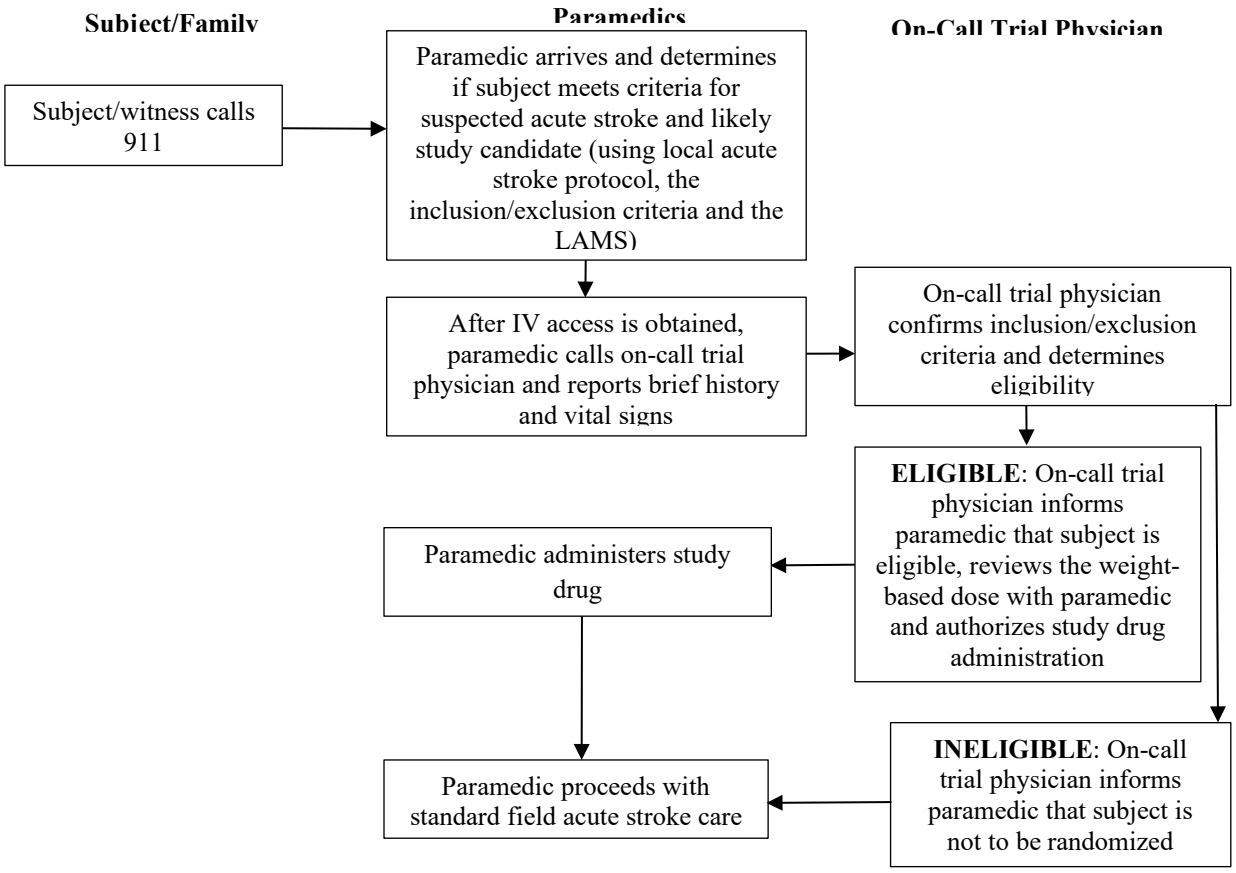
Paramedics will follow their local approved acute stroke protocol (currently the Cincinnati Prehospital Stroke Scale) to determine if the suspected stroke patient is a candidate to be transported directly to a designated stroke center. If the subject is such a candidate and IV access has been obtained, paramedics will then review the brief medical history, vital signs and other additional inclusion/exclusion criteria in consultation with an on-call trial physician to determine if the subject is eligible for the trial. The set of vital signs that were used by the paramedic and the on-call trial physician to determine eligibility for the study will be documented in the eCRF as the first set of vital signs ("Vital signs (time 1)"). The eligibility criteria include items from the mLAPSS, a brief hospital screening instrument designed for use by paramedics to reliably and rapidly identify acute stroke patients, while excluding those with common stroke mimics (e.g., seizure, hypoglycemia) with increased sensitivity[15]. Stroke severity will be graded by the LAMS, to rapidly quantify stroke severity in the field. The LAMS is based on items of facial weakness, arm strength and grip to yield a total 0-5 scale with accuracy comparable to that of the full NIHSS[16, 17]. The paramedic will determine the subject's weight by first asking the patient, second asking a family member, or third by estimating weight[18, 19].

The on-call trial physician will verify the diagnosis of suspected acute stroke and determine the eligibility for study according to the eligibility criteria listed in Section 5. The on-call trial physician will review the weight-based dose with paramedic, approve each subject for randomization and will authorize study drug administration by the paramedic prior to arrival at the ED.

If more than 10 minutes have elapsed between the first set of vital signs and the authorization for study drug infusion, a blood pressure (BP) recording may be taken prior to infusion start provided that, in the paramedic's judgement, this does not interfere with the timeliness of transfer of the subject to the stroke center. If taken, this BP measurement should be performed approximately 10 minutes (but not exceeding 15 minutes) or less prior to infusion start. For the purpose of clarity, if taken, this BP recording will serve solely as a baseline for any additional BP recordings that will be taken after drug infusion in lieu of the BP taken at the time of assessment of eligibility. However, this BP measurement is not used for assessment of eligibility, as the discussion with the on-call trial physician was based on the first set of vital signs. If taken, this pre-study drug infusion BP will be recorded in the eCRF under "Vital signs (time 2)".

Randomization time is defined as the moment a subject receives any amount of study drug. Study enrollment procedures will only begin once the paramedic has successfully obtained IV access and approval by the on-call physician is obtained. The paramedic will insert a safety catheter needle into the subject's arm, secure it, and cap it with a saline lock per standard practice. See Figure 6-1: Field Events Flow Diagram for the field events flow diagram.

Figure 6-2: Field Events Flow Diagram



6.2 Study Drug Preparation and Infusion

Each participating ambulance will be stocked with one single use vial of nerinetide or matching placebo, per local EMS hub working practice. Study drug will be stored at 2 to 8°C in a small, portable refrigerator/cooler placed on the ambulance. Paramedics will also be provided with a FRONTIER case for infusion.

Provided that adequate IV access has been obtained and the on-call trial physician has authorized study drug preparation, the paramedic will remove the vial of study drug from the portable refrigerator/cooler, check the integrity of the vial, the expiration date and the temperature tag, and enroll the subject provided that there are no integrity, expiration or temperature excursion issues.

To authorize study drug preparation the on-call trial physician will refer to the weight-based dosing chart (also in the FRONTIER case for the paramedic) and in conjunction with the paramedic, will determine, based on the patient's reported weight, the volume of study drug to withdraw from the vial and transfer to the 50 mL saline IV mini-bag using aseptic technique (See Table 6-1 for the conversion chart).

The paramedic will then prepare the study drug for infusion by snapping off the study drug syringe vial cap and transferring the appropriate volume of study drug (6-13.5 mL) into the saline IV mini-bag using the standard 20 mL syringe and blunt tip needle and inverting it several times to mix. Next, the Moog Curlin IV infusion administration set will be attached to the saline IV mini-bag using the integrated bag spike and inserted into the pump. The paramedic will connect the tubing to the catheter and select the single pre-programmed infusion rate and volume protocol to begin the 10 ± 1-minute infusion. When the entire content of the saline IV mini-bag is delivered, this will yield a target dose of 2.60 mg/kg (up to a maximum dose of 270 mg).

Treatment will be administered within three hours of symptom onset. After termination of the infusion, the IV tubing will be disconnected from the IV catheter and will be disposed of according to biohazardous waste guidelines. The indwelling IV catheter may be withdrawn or used for other purposes at the discretion of the emergency medical/nursing staff. The study bracelet will be applied to the subject's wrist, for identification of the subject as a research participant, as soon as the study drug infusion begins. After each randomization, the used study drug vial will be retained, and the pump checked and reset as per the local working practice.

Table 6-2: Conversion Chart Mapping Subject's Weight to the Amount of Study Drug Withdrawn into the Syringe (mL) for Transfer to the 50 mL Saline Intravenous Mini-bag

Estimated Weight (kg)		Estimated Weight (lbs)		Syringe Volume (mL) to Transfer to 50 mL Saline IV Mini-bag
Low	High	Low	High	
≥45	≤49	≥ 99	≤108	6
>49	≤54	>108	≤119	7
>54	≤59	>119	≤130	7
>59	≤64	>130	≤141	8
>64	≤69	>141	≤152	9
>69	≤74	>152	≤163	9
>74	≤79	>163	≤174	10
>79	≤84	>174	≤185	11
>84	≤89	>185	≤196	11
>89	≤94	>196	≤207	12
>94	≤99	>207	≤218	13
>99	≤120	>218	≤264	13.5

6.2.1 Early Study Drug Cessation

If any SAE is observed during dosing, dosing shall be immediately terminated. If any moderate or severe AE is observed, the paramedic and/or physician may terminate drug administration at his/her discretion.

6.2.2 Treatment of Hypotension

If hypotension (systolic < 80 mmHg; or any level of decreased blood pressure that the physician deems to be clinically relevant) is observed in a subject, the hospital physician will be instructed,

at his/her discretion, to administer any medication that they deem to be required for the patient's health and safety.

There is no specific treatment requirement related to treating hypotension that may be observed in subjects with stroke who are also receiving nerinetide. Treatment of emergent 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 patients may be observed for spontaneous recovery.

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

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

All subjects will be observed by the trial team at each site for these and other potential complications throughout the clinical trial. All subjects 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 eCRF.

6.2.3 Increases in Histamine

Nerinetide has undergone preclinical testing in rats, dogs, rabbits, and non-human primates. When administered in these animals at doses higher than the one proposed in the current clinical trial, nerinetide has produced apparent anaphylactoid events characterized by histamine release in rats and dogs. The observed signs and symptoms were compatible with the physiological effects of histamine; specifically, transient hypotension and hives. This raises the possibility that anaphylactoid reactions to nerinetide could occur in humans. Patient susceptibility to drug-induced anaphylactoid events is highly variable. Therefore, potentially severe anaphylactoid reactions to the study drug may be encountered. Anaphylactoid reactions, including histamine-related reactions such as urticaria, angioedema, bronchoconstriction, laryngospasm or hypotension, should be monitored. In the event that an anaphylactoid reaction requiring treatment occurs, treatment should follow the same algorithms as would be followed if a spontaneous anaphylactoid reaction occurred in the community.

- Follow emergency medicine ABCs (airway, breathing, circulation) and establish airway first.

- Treat hypotension with crystalloid, colloid and/or vasopressors as needed (see Section 6.2.2).
- Consider treatment with antihistamine agents (diphenhydramine 50 mg IV, ranitidine 50 mg IV) and corticosteroids if the reaction is severe.
- Consider using subcutaneous or IV epinephrine.
- If bronchospasm or laryngospasm are important symptoms, consider treatment with inhaled racemic epinephrine.

6.2.4 Hyperglycemia

In the Phase 1 study, 9% of all subjects receiving any dose of nerinetide (0.02-3.75 mg/kg) had increases in blood glucose, as measured in their laboratory sample. However, in the Phase 2 study, there were no noteworthy differences between the nerinetide and placebo groups in blood glucose across time and at no timepoint did greater than two subjects have a clinically significant abnormal blood glucose result in either treatment group.

In this study, serum electrolytes and blood glucose will be drawn as part of the standard-of-care upon arrival to the receiving acute stroke hospital. If hyperglycemia is noted, the emergency physician will treat the subject on a case-by-case basis. As there is no known relationship between nerinetide administration and hyperglycemia (based on rat, dog, and human studies), there is no unique protocol for blood sugar management following nerinetide administration.

6.3 Post-Randomization

6.3.1 Day 1-ED Arrival Procedures

After the subject is deemed a candidate for redirect and transport to a designated stroke center, an alert will be sent to the receiving stroke center ED per local EMS stroke triage protocol that a stroke subject will be arriving shortly at the receiving stroke center. After the subject is randomized, the on-call trial physician, or attending paramedics per local practice, will alert the study coordinating office and the study nurse/coordinator at the receiving acute stroke hospital that a randomization has occurred. Upon arrival at the ED, the study chart insert will be provided by the paramedic to the receiving ED staff.

The paramedic will take a BP recording immediately following, and no later than 15 minutes after, the termination of the study drug infusion provided that it does not interfere with standard of care. This BP recording will be entered into the eCRF under “Vital signs (time 3)”.

Subjects will receive all standard-of-care treatment for suspected acute stroke both in the prehospital and hospital setting. Upon arrival at the acute stroke hospital, subjects may receive standard-of-care treatments including thrombolytic or endovascular therapy as appropriate. Standard stroke imaging, laboratory assessments, 12-lead ECG and pregnancy testing will also be conducted per standard practice at the receiving acute stroke hospital and analyzed for safety.

The Schedule of Assessments (Table 1-1) lists the study activities.

6.3.2 Consent Process

All sites will request an exception to consent approach to enrolment in this time-sensitive medical emergency setting to address the urgent medical need of patients with suspected acute stroke. This approach will be implemented at the discretion of their supervising Research Ethics Board (REB).

Subjects will then be informed of the study after arrival at the ED and consent will be sought for the remaining follow-up from the subject once they regain capacity or a legally authorized representative (LAR) becomes available. Any information obtained when the researchers were acting on an exception to consent will remain part of the study information. For this study "Acting on an exception to consent" in relation to access to medical records shall take the meaning as defined by FDA regulation and guidance on "Exception from Informed Consent Requirements for Emergency Research"[20], that investigators shall have access to all study information, including the subject's entire medical record, from the moment of enrollment, and until the end of the subject's participation in the study either through completion of the study period, death, withdrawal, or explicit refusal to participate further.

Since subjects in this protocol are enrolled under an exception to consent approach, when a subject or LAR subsequently declines consent, this will be deemed to be a withdrawal of consent. For the purposes of clarity, unless otherwise indicated by a site REB, this study will follow FDA 21 CFR 50.24 guidance on Exception from Informed Consent Requirements for Emergency Research[20] which states:

- "In general, the investigator should arrange to have access to all of the records that are generated and maintained from enrollment until discharge or death, unless the subject or the subject's legally authorized representative (LAR) or family member discontinues the subject's participation in a study" (Section 110).
- "If a subject or the subject's LAR discontinues the subject's participation in the study, the investigator would continue to have access to data that have already been collected" (Section 111).
- "However, the investigator would not have access to the subject's medical records after the date of discontinuation (even if the subject has not been discharged from the hospital), unless the subject or the subject's LAR specifically consents to such access" (Section 111).
- "A subject may not withdraw use of his or her data that have already been collected. FDA regulations require investigators to prepare and maintain adequate case histories recording all observations and other data pertinent to the investigation on each individual treated with the investigational product. If a subject were to be able to dictate whether already collected data are included or excluded, the potential for bias would be immense, particularly if the clinical investigation were not blinded" (Section 112).
- This is further supported by FDA guidance document FDA 21 CFR UCM126489, *Data Retention When Subjects Withdraw from FDA-Regulated Clinical Trials*[21] which states: "FDA recognizes that a subject may withdraw from a study; however, the withdrawal does not extend to the data already obtained during the time the subject was enrolled. FDA's longstanding policy has been that all data collected up to the point of withdrawal must be maintained in the database and included in subsequent analyses, as appropriate"

If repatriation to another hospital or discharge occurs, the research nurse/coordinator will visit the subject in person (or by telemedicine or phone) to obtain all protocol assessments.

6.3.3 Day 2-24-Hour Visit

The Qualified/Principal or Sub-Investigator or nurse/coordinator will personally visit the subject to discuss the trial and review the implementation of study procedures as soon as possible, preferably within 24 hours of arrival to the acute stroke hospital. During this contact/visit site staff will complete the protocol specific activities:

- Attempt to obtain consent from the subject or LAR (see Section 6.3.2).
- Pre-morbid mRS
- Assessment if stroke symptoms have resolved

In addition, site staff will review the results from standard of care treatment as recorded in the subject medical records and collect the following data (select data from the closest timepoint to 24 hours):

- Complete medical history
- Prior medications
- Vital signs including blood pressure, heart rate, temperature and SaO_2,
- Laboratory test results (as per local hospital standard of care) including CBC, electrolytes, creatinine, glucose, INR and PTT and
- Any SAEs, AE and medications reported since arrival in the hospital
- NIHSS

6.3.4 Day 4 Visit

During this visit qualified site staff will complete the following protocol specific activities:

- Attempt to obtain consent from the subject or LAR if not already obtained (see Section 6.3.2).
- Actual weight
- NIHSS
- mRS
- Barthel Index

In addition, site staff will review the results from standard of care treatment as recorded in the subject medical records or in person interview (See Section 8.2) and collect the following data:

- Vital signs including blood pressure, heart rate,
- Any SAEs, AE and medications reported since arrival in the hospital
- Note: the mRS at discharge should also be collected

6.3.5 Day 30 Visit or Contact

At Day 30 it is preferred that participants will return to clinic. If an in person visit is not possible the participant can be contacted by telemedicine (preferred) or by telephone (last option). During this visit/contact qualified site staff will complete the following protocol specific activities:

- Attempt to obtain consent from the subject or LAR if not already obtained (see Section 6.3.2).
- mRS
- Barthel Index
- EQ-5D-5L

- Collect any AE, SAE and medication in the treatment of any SAEs since the Day 4 contact (see Section 8.2).

6.3.6 Day 90 Visit

At Day 90 it is preferred that participants will return to clinic. If an in person visit is not possible the participant can be contacted by telemedicine (preferred) or by telephone (last option). During this visit, qualified site staff will complete the following protocol specific activities:

- attempt to obtain consent from the subject or LAR if not already obtained (see Section 6.3.2).
- mRS
- NIHSS
- Barthel Index
- EQ-5D-5L
- ALDS
- Collect any SAE occurring since the Day 30 contact (see Section 8.2).

6.4 Subject Withdrawal Criteria

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

- AEs (as determined by the Qualified/Principal or Sub-Investigator)
- Administrative reasons (uncooperative, noncompliant, etc.)
- Subject's decision not to participate any further
- If it is in the subject's best interest, per the Qualified/Principal or Sub-Investigator

If the subject or LAR withdraws consent, subject 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 eCRF. Otherwise, all randomized subjects will continue to be followed according to protocol requirements and follow-up data will be included in the analysis. Criteria for removal of subjects will be recorded and reported.

In the event a subject is lost-to-follow up, all efforts made by the nurse/coordinator to bring the subject in for a clinic visit or to undertake a home visit for follow-up will be documented.

6.5 Prior and Concomitant Medications

There are no prior medications that are exclusionary. Subjects will receive standard-of-care, including thrombolytic or endovascular therapy, as appropriate, upon arrival at acute stroke hospital. A separate IV line should be employed if tPA is being infused.

All medications taken within three days of treatment initiation will be considered prior medications and will be reported in the eCRF.

Concomitant medications (Conmeds) will be collected from the time of randomization to the Day 4 Visit/contact. Conmed identification maybe collected via acute stroke hospital patient records or verbal histories from the subject or LAR. Conmeds received while the subject is admitted to the acute stroke hospital will be identified from both hospital records and verbal histories.

After discharge from the acute stroke hospital and for the Day 4 visit, the subject (or LAR if the subject is not able to respond to the questions) will be asked about medications taken since the last contact. Conmeds that were ongoing at the last contact will be updated with a stop date or confirmed as ongoing at Day 4.

7 ASSESSMENT OF EFFICACY

7.1 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 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. mRS scores range from 0 to 6, with 0 indicating no residual symptoms; 5 indicating bedbound, requiring constant care; and 6 indicating death. Since substantial interobserver variability occurs when raters perform mRS scoring intuitively,[22, 23] it is advisable to use a formal, operationalized method for mRS scoring. This trial will use the Rankin Focused Assessment (RFA) to guide Rankin Scale scoring. The RFA is a validated, systematic, structured assessment tool to guide raters in assigning mRS grades. It has excellent inter-rater reliability in assigning final outcome mRS disability ratings to patients three months post stroke[24-26]. It was developed by selecting and refining elements from prior instruments such as the Structured Interview and video training which have been found to only moderately improve mRS reliability[27]. It consists of a 4-page form accompanied by a 5-page instruction sheet. It takes 3-5 minutes to apply and provides clear, operationalized criteria to distinguish the seven assignable global disability levels. The assessment permits and encourages the rater to gather data from all available useful sources. It rates the patient based on current actual capacity and performance and not specifically stroke-related dysfunction[26].
The mRS will be obtained via the RFA on acute stroke hospital discharge (in person or by medical chart review), Day 4 (in person), 30 (in person, by telemedicine, or by phone), and 90 (in person, by telemedicine, or by phone). Premorbid mRS status will also be obtained at 24 Hours. The mRS will only be administered by those trained and certified in the use of this scale.

7.2 The National Institutes of Health Stroke Scale

The NIHSS is a standardized neurological method found to be a valid and reliable measure of disability and recovery after acute stroke[27]. 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 shortly after arrival in the ED (no more than four hours post-dose), at 24 Hours, Day 4 and 90 by the Investigator or stroke center nurse/research coordinator. The NIHSS will only be administered by those trained and certified in the use of this scale.

7.3 Barthel Index

The Barthel Index is an index of functional independence[28] that has been found to be a valid measure of activities of daily living when employed in stroke trials[29]. Barthel Index scores range from 0 to 100, with higher scores indicating greater independence in activities of daily living and mobility. The Barthel Index will be administered on Day 4 (in person), 30 (in person or by telemedicine/phone) and 90 (in person or by telemedicine/phone) by those trained in the use of these scales.

7.4 Mortality Rates

Mortality rates are defined as the number of deaths observed divided by the number of subjects observed over the 90-day study period.

7.5 Worsening of Stroke

The rates of worsening of stroke, defined as the proportion of participants with a worsening of their strokes over the 90-day study period in the nerinetide and placebo control subjects. Worsening of stroke is defined as progression, or hemorrhagic transformation, of the index stroke as documented in the study CRF that (i) is deemed life-threatening and/or (ii) results in increased disability as gauged by a ≥4 point increase from lowest NIHSS during hospitalization and/or (iii) results in death.

7.6 The 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[30]. Each dimension has five response categories corresponding to: no problems, slight, moderate, severe and extreme problems[31]. The instrument is designed for self-completion (or by proxy), and respondents also rate their overall health on the day of the interview on a 0–100 hash-marked, vertical visual analogue scale (EQ-VAS). The EQ-5D-5L will be administered on Day 30 (in person, by telemedicine, or by phone) and 90 (in person, by telemedicine, or by phone).

7.7 Return to Baseline Function with 24 Hours

The number of randomized subjects whose symptoms fully return to baseline function within 24 hours of symptom onset will be calculated based on whether or not the subject's final diagnosis was acute cerebral ischemia and the subject's stroke symptoms returned to baseline within 24 hours of stroke symptom onset. A subject will be considered returned to baseline if the subject meets any one of the following criteria:

1) Diagnosis of TIA
2) Any stroke diagnosis with a 24h NIHSS =0

7.8 Subjects Who Receive Reperfusion Therapy

The number of randomized subjects who receive reperfusion therapy will be calculated based on use and type of recanalization therapy received (tPA, TNK, endovascular treatments).

7.9 Subjects Who Receive Thrombolysis

The number of randomized subjects who receive thrombolysis will be calculated based on use of any thrombolysis (tPA, TNK).

7.10 Academic Medical Center Linear Disability Score

Selected items in the ALDS item bank will be formed into five 15- item sets, following the method proposed by Saver (personal communication), based on the method by Weisscher[32]. The

appropriate 15-item set will be administered to the individual subject according to mRS score as measured by the RFA at the Day 90 visit.

8 ASSESSMENT OF SAFETY

8.1 Adverse Event Definitions

The following definitions are taken from the ICH E2A Guideline for Clinical Safety Data Management: Definitions and Standards for Expedited Reporting.

Adverse Event:

An AE is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment. An AE can therefore be any unfavourable and unintended sign (including clinically significant abnormal laboratory findings, for example), symptom or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

All AEs include serious and non-serious AEs.
Pre-existing medical conditions are not to be reported as AEs. However, if a pre-existing condition worsens in frequency or intensity, or if in the assessment of the treating physician there is a change in its clinical significance, this change should be reported as an AE (exacerbation). This applies equally to recurring episodes of pre-existing conditions (e.g., asthma) if the frequency or intensity increases post-randomization.

Adverse events of special interest (AESIs) will be defined as AEs related to hypotension, angioedema, or anaphylactoid reactions that occur within the first two hours following the end of study drug administration.

NOTE: Any diagnosis or findings identified by CT/CTA and/or by the treating physician after arrival to hospital that is/are deemed by the treating physician(s) to be causally related to the diagnosis of suspected stroke made at the time of enrollment shall not be treated as a new AE or SAE, but rather as a pre-existing condition and will be reported in the medical history. For example, the diagnosis of an atherosclerotic plaque in the carotid artery in a subject with an ischemic stroke. All newly-diagnosed underlying causes of stroke mimicking conditions, not wholly represented by the diagnosis for the study qualifying suspected stroke, shall be treated as a new AE or SAE (e.g. brain tumor). Any worsening, transformation, change or treatment of the initial findings shall be considered as a new AEs/SAEs.

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 products" 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.

AE severity and relationship per the definitions presented in Table 8-1 will also be assessed.

Table 8-3: Definitions of AE-Related Terms

10. AE Severity	
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.

AE Relationship	
Probably:	A clinical event, including laboratory test abnormality, with a reasonable time sequence to drug administration, unlikely to be attributed to concurrent disease or other drugs or chemicals, and which follows a clinically reasonable response on withdrawal.
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.
Unlikely*:	A clinical event, including laboratory test abnormality, with a temporal relationship to drug administration which makes a causal relationship improbable, and which other drugs, chemicals or underlying disease provide plausible explanation.
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.

* The term 'unlikely' will no longer be used to assess AE relationship to study drug for any AEs/SAEs with a Start Date after local REB approval for Protocol Amendment #4.

Serious Adverse Event:

Serious and *severe* are not synonymous. The term "severe" is often used to describe the intensity (severity) of a specific event (as in mild, moderate, or severe myocardial infarction); the event itself, however, may be of relatively minor medical significance (such as severe headache). This is not the same as "serious," which is based on patient/event outcome or action criteria usually associated with events that pose a threat to a patient's life or functioning. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations.

An SAE is defined as an AE which:

1. Results in death
2. Is life-threatening
3. Requires or prolongs inpatient hospitalization
4. Results in persistent or significant disability/incapacity, or
5. Is a congenital/birth defect

An important medical event may also be deemed an SAE, based upon the medical judgment of the Qualified/Principal Investigator or Sub-Investigator, Medical Monitor, or Sponsor, when it may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above [e.g., incidental brain tumor discovered on computerized tomography (CT) scan].

8.2 Identification of Adverse Events

AE monitoring and reporting will be followed-up until Day 30. SAEs will be followed through the final study exit visit (Day 90 Visit or death or end of study whichever is sooner) or until the subject is deemed "lost to follow-up".

AE identification while the subject is admitted to the acute stroke hospital will be collected via acute stroke hospital patient records and verbal histories from the subject or LAR. For follow up visits after discharge from the acute stroke hospital the subject (or LAR if the subject 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.

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.

A consistent methodology of eliciting AEs at all subject 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?

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.

AEs should be reported by the site to the Sponsor as they occur on the eCRF. Documentation must be supported by an entry in the subject's file.

8.3 Reporting of Adverse Events

AEs should be reported as they occur on the eCRF. Documentation must be supported by an entry in the subject's file. Each event should be described in detail along with start and stop dates, severity, relationship to investigational product as judged by the Investigator, action taken and outcome.

Additional information may be requested by the Sponsor from the site Investigator in connection with any AEs of Special Interest (AESIs) related to hypotension, angioedema, or anaphylactoid reactions (as derived from MedDRA preferred terms and Standardised MedDRA Queries (SMQs)) and occurring within the first two hours of study drug administration.

8.4 Reporting of Serious Adverse Events

In order to comply with current regulations on SAE reporting to health authorities, the Investigator must document all SAEs regardless of causal relationship and notify the Sponsor. The Investigator will give access and provide the Sponsor with all necessary information to allow the Sponsor to conduct a detailed analysis of the safety of the investigational product. It is the responsibility of the Investigator to request all necessary documentation (e.g., medical records, discharge summary, autopsy) in order to provide comprehensive safety information. All relevant information must then be transcribed into the e-SAE Form.

8.4.1 Reporting by the Investigator

All SAEs must be reported to the Sponsor immediately after the local Investigator's first awareness of its occurrence. SAEs will be reviewed by the Pharmacovigilance Department at NoNO Inc. and the Medical Monitor. The Investigator will report the SAEs using the SAE form provided by the Sponsor. The completed SAE form should be sent to:

Pharmacovigilance at NoNO Inc.
sae@nonoinc.ca (for email) or Tel: *Redacted- Personal data*

8.4.2 Reporting SAEs to Health Canada and REBs

The Sponsor will inform the health authorities of any reportable SAEs according to their regulatory requirements. Reporting to the health authorities will be according to the Sponsor's standard operating procedures.

SAEs that are assessed by the Sponsor to be unexpected and related to study drug (expedited reporting SAEs) will be reported to Health Canada as per country requirements. All other SAEs will be reported to regulatory agencies based upon local reporting requirements.

The Sponsor's Medical Monitor or designee will notify the Investigators in writing of the occurrence of any reportable SAEs. The Investigators will be responsible for informing the Research Ethics Boards as per their local requirements.

8.5 Vital Signs

In the ambulance, paramedics will measure vital signs per standard practice. This includes vital signs for inclusion/exclusion and blood pressure within 15 minutes of randomization (if within 15 minutes may only be one set of vitals). A final blood pressure reading will be taken no later than 15 minutes after study drug infusion is completed provided that it does not interfere with standard of care.

On ED arrival, blood pressure, heart rate, temperature and oxygen saturation (SaO_2) will be recorded per standard-of-care. The assessment of the set of vital signs closest to the 20-minute time-point after ED arrival will be entered into the eCRF. Blood pressure, heart rate, temperature and SaO_2 will also be taken at 24 Hours per standard of care.

Actual weight will be measured in hospital within four days. If actual weight cannot be measured due to, for example severe illness, weight will be determined by first asking the subject, second asking a family member or third by estimation. The method of determining weight will be entered into the eCRF.

On Day 4, blood pressure and heart rate will be recorded.

Clinically significant abnormalities in vital signs as deemed by the local Investigator (or delegate) will be recorded as AE/SAEs

8.5.1 Hypotension

If hypotension occurs, the hypotension is to be recorded as an AE in the eCRF.

8.6 Biochemistry and Hematology

Complete blood count (CBC), electrolytes, serum creatinine, glucose, international normalized ratio (INR) and prothrombin time (PTT) will be collected per-standard of care upon arrival at the ED and at 24 Hours.

Clinically significant abnormalities as deemed by the local Investigator (or delegate) will be recorded as AE/SAEs

8.7 Follow Up and Reporting of Pregnancies

Known or presumptive signs of pregnancy, or breastfeeding, is an exclusion criterion for enrolment in this study, but a subject could potentially be or become pregnant during her participation. All pregnancy cases should be reported if they occurred during the study. To report the pregnancy case, the Investigator must fill out a Pregnancy Reporting Form and inform the Sponsor as soon as possible upon identification of the pregnancy. Study staff must then maintain contact with the subject to obtain information about the outcome—i.e., details about the delivery and the newborn, or about pregnancy termination—and must update the Pregnancy Reporting Form. This information should be provided to the Sponsor within one month of delivery.

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).

8.8 12-Lead Electrocardiogram Monitoring

On ED arrival, a 12-lead ECG will be performed per standard-of-care and the following data will be recorded in the eCRF: Ventricular rate, PR interval, QRS duration, QT, QTc, P-axis and QRS-axis. Clinically significant findings in 12-lead ECG as deemed by the Investigator or delegate will be recorded on the eCRF as AEs.

8.9 Symptomatic Hemorrhagic Transformation, Worsening Stroke and Recurrent Ischemic Stroke

The presence of symptomatic hemorrhagic transformation, worsening of existing stroke and recurrent ischemic stroke will also be assessed and recorded as applicable in the AE or SAE form of the eCRF.

The symptomatic hemorrhagic transformation of cerebral infarct will be defined as central nervous system hemorrhage in a patient with an entry CT or MRI scan negative for hemorrhage, and either (1) a haemorrhage appearing in the area of the qualifying stroke and deemed by the treating physician to be causally related to the neurological deterioration, or (2) appearing in a different vascular territory than the qualifying stroke and deemed by the treating physician to be causally related to a new neurological deficit[33]. Worsening of existing stroke is defined as a clinical, progressive deterioration occurring after study day 1 but before study day 5, without intracerebral haemorrhage or other non-ischemic cause for symptoms and attributable to the entry infarct territory. Recurrent ischemic stroke is defined as a clinical, sudden, and persisting (> 24 hours) deterioration occurring without intracerebral hemorrhage or other non-ischemic cause for

symptoms, and (1) attributable to a newly involved territory at any time during the study, or (2) attributable to the entry infarct territory but occurring after study day 5[34, 35]. The Sponsor will adjudicate all potential occurrences of these AEs.

8.10 Other Assessments

The following additional information will be reported:

- Date and time of stroke symptom onset, defined as the time last seen in a usual state of health and time of start of study drug administration.
- The accuracy of estimated patient weight determination by the paramedic versus actual patient weight measured in hospital
- Proportion of subjects randomized with the final diagnosis of acute cerebral ischemia
- Proportion of subjects randomized by discharge destination from acute stroke centre (e.g., home, rehabilitation center)

9 Statistics

9.1 Sample Size Considerations

The primary outcome variable is the overall proportion of subjects experiencing a favourable functional outcome 90 days post randomization. Sample size projections assume, based on the Field Administration of Stroke Therapy – Magnesium (FAST-MAG) experience[34, 36], that approximately 72% of randomized subjects will have AIS, 24% will have intracerebral hemorrhage as their stroke subtype, 4% will have stroke-mimicking conditions, and that treatment benefit is obtained mainly in patients with acute cerebral ischemia. Assuming a 26% overall responder rate for the placebo group using the sliding dichotomy definition of responder (Table 9-1), there will be an estimated 80% power to detect a 12% absolute effect difference between response rate (proportion of responders) with nerinetide and placebo, at alpha level 0.05, 2-sided with a planned sample size of 506 evaluable subjects, randomized 1:1, 253 per group. The 12% absolute response rate difference is judged to be the minimally clinically important difference to justify prehospital administration of nerinetide; it was selected in part because it is similar in magnitude to a previously proven clinically important difference: The absolute response rate to tPA[37]. The sample size will be inflated 10% to account for loss-to-follow-up and drop-outs. (n=558, 279 per group).

If loss-to-follow-up and drop-outs exceeds 10% (more than by the time that original enrollment reaches approximately 80%), the sample size may be inflated an additional 5% (n = 586, 293 per group).

9.2 Analysis Populations

FRONTIER subjects consist of a number of important analysis populations for primary, secondary, or exploratory analyses. The analysis populations and corresponding analyses in the study are summarized in Table 9-1.

9.2.1 Modified Intent-to-Treat Population

The modified intent-to-treat (mITT) population is defined as all subjects receiving any amount of study drug, with at least one post-dose mRS assessment. Deceased subjects will be included in the mITT population with an mRS score of 6, NIHSS of 42 and Barthel Index of 0.

9.2.2 Primary Efficacy Analysis Population

This is a defined by the stepwise process described in Section 9.3.1. This process ensures that the analysis plan is aligned with knowledge gained from the ESCAPE-NA1 trial about the effect modifiers discussed in Section 2.6.

9.2.3 Per-Protocol Population

The Per-Protocol population comprises all subjects in the mITT population without major protocol deviations. A “major protocol deviation” is defined as those with the potential to bias, confound, or otherwise obscure the treatment effect estimates or which involve ethical standards. Major protocol deviations will be reviewed per subject during a blinded data review meeting prior to database lock and unblinding and may result in the subject being removed from the Per-Protocol

analyses. Deceased subjects will be included in the Per-Protocol population with an mRS score of 6, NIHSS of 42 and Barthel Index of 0.

9.2.4 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.

9.2.5 Confirmed Acute Cerebral Ischemia Population

The Confirmed Acute Cerebral Ischemia (CACI) population will be defined as subjects in the mITT population who are deemed by the treating stroke physician as having sustained a diagnosis of acute ischemic stroke of any severity or a transient ischemic attack. Subjects who are diagnosed with a hemorrhagic stroke or with an alternate diagnosis of a stroke-mimicking condition including but not limited to brain tumor, seizure, hemiplegic migraine or conversion reaction will not be deemed to have acute cerebral ischemia and will not be included in this subset. This analysis will be performed in a similar manner to the primary analysis.

9.2.6 Subjects Treated with a Reperfusion Therapy Population

The Subjects Treated with a Reperfusion Therapy population will be defined as those subjects in the mITT population who were documented in the CRF as having received thrombolysis, EVT, or both within 24 hours of hospital arrival.

9.2.7 Subjects treated with Thrombolytics Population

The Subjects Treated with Thrombolytics population will be defined as those subjects in the mITT population who were documented in the CRF as having received thrombolysis with alteplase or tenecteplase within 24 hours of hospital arrival.

9.2.8 Confirmed Intracerebral Hemorrhage (ICH) Population

The Confirmed ICH analysis population will be defined as those subjects in the mITT population who were documented in the CRF as having a discharge diagnosis of “intracerebral hemorrhage”

Other subgroups of interest are described in Section 9.7.

Table 9-4: Analysis Populations with Efficacy and Safety Outcome Measures

							Subjects without reperfusion therapy[3]			
	Safety	**PP[1]**	**mITT[1]**	**Confirmed acute cerebral ischemia (CACI)[2]**	**Subjects with reperfusion therapy[1]**	**Subjects treated with thrombolytics[2]**	**All Subjects without reperfusion therapy**	**CACI without reperfusion therapy**	**Confirmed ICH**	**Other**
Primary Efficacy Outcomes										
mRS Responder		X	X	X[2]	X	X[2]	X[2]	X[2]	X[3]	X[2]
Secondary Efficacy Outcomes										
mRS Shift			X	X[2]	X	X[2]	X[2]	X[2]	X[3]	X[2]
Mortality			X	X[2]	X	X[2]	X[2]	X[2]	X[3]	X[2]
Worsening of Stroke			X	X[2]	X	X[2]	X[2]	X[2]	X[3]	X[2]
NIHSS (0-1 vs >1)			X	X[2]	X	X[2]	X[2]	X[2]	X[3]	X[2]
Barthel Index			X	X[2]	X	X[2]	X[2]	X[2]	X[3]	X[2]
Tertiary Efficacy Outcomes										
mRS 0-2 and 0-1			X		X					
EQ-5D-5L			X		X					
Return to baseline within 24 hours			X		X					
% Received Reperfusion Therapy			X		X					
% Received Thrombolysis			X		X					
mRS Responder at Day 30			X		X					
ALDs[2]			X[2]		X[2]					
SAFETY										
# SAEs				X[2]	X[2]				X[2]	
Mortality	X-All			X[2]	X[2]				X[2]	

[1]Analysis will be done for the primary efficacy analysis population for efficacy as determined by the interaction effect and proportionality tests per Section 9.3.1.
[2]Analysis to be conducted only if efficacy outcomes warrant further exploration
[3]Analysis to be conducted only if the 'Subjects with Reperfusion" population is the primary efficacy analysis population

9.3 Efficacy Analysis

The analysis populations and corresponding efficacy analyses are summarized in Table 9-1.

Summary statistics will be presented. For continuous endpoints, the summary statistics will generally include: number of subjects with data, mean, standard deviation, median, quartiles, and range. For categorical endpoints, the summary statistics will generally include: number of subjects in corresponding analysis population, number and percentage of subjects in each category.

9.3.1 Analysis of Primary Efficacy Endpoint

The primary efficacy outcome is the overall proportion of subjects experiencing a favorable functional outcome 90-days post-randomization; subjects who are responders as defined in Table 9-2 are said to have favorable functional outcomes.

The primary hypothesis that administration of nerinetide will result in a higher rate of responders will be tested using a generalized linear model, adjusted for EMS hub, age and LAMS score, with log link to directly estimate RR, consistent with 2010 recommendations to avoid overestimation of treatment effects via odds ratios[38],[3] (This is to provide the best treatment effect estimate of the absolute difference in the primary outcome variable as responses are expected to be relatively common and a direct odds ratio may overestimate the RR, and hence the absolute treatment effect, in such a model.) If a binomial model fails to converge using a log link, the plan will be to revert to traditional logistic regression using a logit link function. Only main effects will be evaluated.

Table 9-5: Sliding Dichotomy Definition of Responder

	Prehospital LAMS 2-3	**Prehospital LAMS 4-5**
Age 79 or under	mRS 0-1	mRS 0-2
Age 80 or over	mRS 0-2	mRS 0-2

As summarized in Section 2.6 a potentially important modifier of the effect of nerinetide is that of reperfusion of the ischemic territory. To align the FRONTIER analysis for this possibility, the following steps will be implemented in order to define the primary efficacy analysis population:

Step 1

Assessment of Interaction Between Treatment and use of Reperfusion Therapy in the mITT population. This will occur using a model that includes the covariates described for the primary efficacy analysis above (EMS hub, age, baseline LAMS score) as well as Reperfusion Therapy (yes/no) and the two-way interaction term for treatment and use of Reperfusion Therapy.

A) If the p-value of the interaction term, derived from a likelihood ratio test, between treatment with nerinetide and use of Reperfusion Therapy is > 0.3 for the drug group versus placebo, the primary efficacy analysis population will include the entire mITT population.

B) If the p-value of the interaction is ≤0.3, Step 2 below will be taken to report balance between the drug and placebo groups in the use of reperfusion therapies in the first 24 hours after hospital arrival.

Step 2

Report balance between the drug and placebo groups in the use of Reperfusion Therapies. This step is necessary because the use of reperfusion therapies is a post-randomization event. The proportion of enrolled subjects treated with a Reperfusion Therapy in nerinetide group compared to the same proportion in the placebo control group will be evaluated using a Fisher's exact test.

A) If the p-value for the difference in proportion of use of Reperfusion Therapy among drug and placebo subjects is > 0.05, nerinetide and placebo groups will be deemed to be balanced with respect to this variable. In such instance, the primary efficacy analysis population will include only subjects who receive Reperfusion Therapy. A supportive analysis to the primary analysis will be conducted separately on the stratum of subjects who did not receive a Reperfusion therapy. Only main effects will be presented.

B) If the p-value for the difference in proportion of use of Reperfusion Therapy among drug and placebo subjects is ≤ 0.05, the nerinetide and placebo groups will be deemed to be unbalanced. In such an instance, the primary efficacy analysis population will be the entire mITT population. The primary efficacy analysis will be supported with a further analysis in which the main effect GLM adjusting for EMS hub, age, baseline LAMS score will also adjust for Reperfusion Therapy (yes/no). Only main effects will be presented.

The primary efficacy will be conducted on the primary efficacy analysis population at the 2-sided 0.05 significance level overall for the trial.

Additional supportive analyses using the primary outcome variable will be performed as indicated for the sub-populations as indicated in Table 9-1, provided that such additional analyses are deemed to be warranted.

9.3.2 Analysis of Secondary Efficacy Endpoints

All secondary analyses in this section will be conducted and presented using the approach outlined above for the primary efficacy analysis population and separately as a supportive analysis in the remaining populations. The fixed sequential order for testing in the defined efficacy population is as specified in the order presented in Table 9-1 and described below.

9.3.2.1 Modified Rankin Scale- Shift Analysis

A shift to reduced functional dependence analyzed across the whole distribution of scores on the mRS at Day 90 or the last rating will also be conducted. This shift will be analyzed using 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 or last rating, relative to the mRS distribution of the placebo subjects. The magnitude of the shift will be estimated as the common odds ratio (95% C.I.). mRS scores of 5 and 6 (bed-bound with severe

disability, and death) will be collapsed into a single category representing severely limited functioning.

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9.3.2.2 Mortality Rate

Mortality rates, as 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, will be analyzed by the same regression model as in the primary efficacy.

9.3.2.3 Worsening of Stroke

The rates of worsening of stroke, defined as the proportion of participants with a worsening of their strokes over the 90-day study period in the nerinetide and placebo control subjects, will be analyzed by the regression method as described for the primary efficacy analysis.

9.3.2.4 National Institutes of Health Stroke Scale

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

9.3.2.5 Barthel Index

The Barthel Index 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 compared using the same logistic regression model as in the primary efficacy.

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9.3.3 Analysis of Tertiary Efficacy Endpoints

Summary statistics for each tertiary efficacy endpoint will be tabulated by treatment group. The tertiary analyses will be considered exploratory and will be conducted in the analysis populations defined in the SAP, if deemed appropriate.

9.3.3.1 Proportion of Subjects with Day 90 mRS ≤ 1 and mRS ≤ 2

The mRS scores will be dichotomized into a) 0-2 (indicating freedom from dependence) versus > 2 (indicating otherwise) and b) 0-1 (indicating freedom from disability) and > 1 (indicating otherwise). The proportion of subjects with freedom from dependence/disability at Day 90 in nerinetide versus placebo control subjects will be compared using the same regression model as in the primary efficacy.

9.3.3.2 EQ-5D-5L

For the EQ-5D-5L, the difference between nerinetide and placebo control subjects in the distribution of the index and VAS scores on these scales at Day 90 will be presented descriptively and analyzed by analysis of covariance (ANCOVA), with EMS hub as stratification factor.

9.3.3.3 Return to Baseline Function within 24 Hours

The proportion of subjects whose symptoms fully return to baseline function within 24 hours of symptom onset in nerinetide versus placebo control subjects will be compared using the same regression model as in the primary efficacy. Return to baseline includes 1) Diagnosis of TIA or 2) Diagnosis of stroke with an NIHSS of 0 at 24 hours.

9.3.3.4 Subjects who Receive Reperfusion Therapy

In the event that the primary efficacy analysis population is the mITT population, the number of randomized subjects who receive recanalization therapy will be calculated based on use and type of reperfusion therapy (tPA, TNK, endovascular treatments). Treatment groups will be compared using the regression model as described for the primary efficacy analysis

9.3.3.5 Subjects Who Receive Thrombolysis

The number of randomized subjects who receive thrombolysis will be summarized. Treatment groups will be compared using the regression model as described for the primary efficacy analysis.

9.3.3.6 Favourable Outcome at 30 days

The proportion of responders at 30 days post-randomization will be analyzed using the logistic regression model as described for the primary endpoint. A responder will be determined based on the sliding dichotomy in Table 9-1.

9.3.3.7 Academic Medical Center Linear Disability Score

For the ALDS, the difference between NA-1 and placebo control subjects in the distribution of scores on the scale at Day 90 will be presented descriptively and analyzed by ANCOVA, adjusting for EMS hub, age, and baselines LAMS score.

9.3.4 Subgroup Analyses

In addition to the analyses described in Sections 9.3.1, 9.3.2 and 9.3.3, additional exploratory subgroup analyses will also be performed if warranted to determine the potential roles of common baseline characteristics and assess potential heterogeneity of treatment effect across subgroups of the mITT population. Specific subgroups of interest include subjects treated with EVT, , treatment with recanalization therapy (tPA, TNK and/or endovascular recanalization), the very elderly (age > 80 years and >75 years of age), men vs women, race and ethnicity, time from stroke onset to randomization, and baseline stroke severity based on LAMS and NIHSS. Further details of the exploratory sub-populations and outcome measures, including the analysis methods, will be specified and detailed as necessary in the Statistical Analysis Plan.

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9.3.5 Handling of 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. Deceased subjects will score 6 on the mRS, 42 on the NIHSS, and 0 on the Barthel Index and will be counted as non-responders. Other missing data will be imputed as described in the Statistical Analysis Plan and, where appropriate, supported by additional imputation methods (e.g., Single imputation, multiple imputation, worst score, best score, 2-way tipping point imputations) where it can be reasonably assumed that data are missing at random. Where data cannot be assumed to be missing at random, no imputation will be undertaken. Sensitivity analyses will assess the effect of best and worst outcome score imputation to assess its impact on the data. Irrespective of the Primary Efficacy Analysis Population, all imputations for missing data will be conducted on the mITT population.

9.4 Analyses of Safety

The assessment of safety will be conducted in all subjects who received any amount of study drug. The main analyses will be frequency of SAEs and 90-day mortality.

9.4.1 SAEs

Event rate (%) for SAEs over the 90-day study period between nerinetide and placebo control subjects will be compared by logistic regression similar to the that of the primary analysis. The frequencies and incidences of SAEs 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 SAEs and discontinuations due to SAEs occurring in subjects in the nerinetide and placebo control groups will be summarized within treatment group. All SAEs will be provided in a listing.

9.4.2 Mortality

Event rate (%) for mortality over the 90-day study period between NA-1nerinetide and placebo control subjects will be compared by logistic regression. The logistic regression model will include factors for treatment group and the EMS hub stratification factor. All deaths will be provided in a listing.

9.4.3 AEs

Additional analyses will consider the frequency of AEs, AESIs and discontinuations due to AEs. AEs will be summarized by presenting, for each treatment group, the number and percentage of subjects having any AE, having an AE in each body system and preferred term. Severity and relatedness to study medication will be recorded according to Table 8-1: Definitions of AE-Related Terms. 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) and for Preferred Term (PT). Summary tables of AESIs (with a start time within 0-2 hours of study drug administration) will also be summarized by MedDRA SOC and PT.

9.4.4 Vital Signs

The maximum deviation of blood pressure from pre-dose between drug and placebo control groups (systolic and diastolic) to 24 hours will be analyzed using ANCOVA, including factors for treatment group, EMS hub, and treatment with intravenous tPA.

9.4.5 Laboratory and 12-Lead ECG Results

Absolute values for laboratory and 12-lead ECG results will be documented descriptively. Clinically significant laboratory and 12-lead ECG abnormalities will be classified as AEs.

9.4.6 Prior and Concomitant Medications

Prior and concomitant medications will be analyzed descriptively. Prior and concomitant medications will be summarized within treatment group using the World Health Organization (WHO) Drug classification.

9.5 Independent Data Monitoring Committee

An IDMC comprising three individuals (e.g., stroke neurologist, neurosurgeon and one statistician) will evaluate the safety data arising from this study. The members of this IDMC will make recommendations to the Sponsor about the continuation or modification of the trial or suspension of the clinical study, according to a written, signed charter which describes the roles and responsibilities of the IDMC and outlines the plan for review of study data.

The IDMC will perform periodic safety reviews of the clinical data. The first reviews will occur once 25, 50 and then 300 subjects have reached their 90-day study visit. Safety reports will include cumulative summary statistics; subject status in the study (e.g., number completed Day 90 visits); baseline characteristics; safety data, including AEs and SAEs by AE code, severity, and relatedness to the study medication; and discontinuations due to AEs.

During these reviews, the IDMC will confirm the safety of the dosing and consent approach. The IDMC will also comment on the balance of nerinetide: placebo subjects, demographics and other data quality and timing metrics during the trial.

10 Direct Access to Source Data/Documents

The Sponsor will be permitted to visit the study facilities at any reasonable time in order to maintain current, detailed knowledge of the study through review of the records, source documents, observation, and discussion of the conduct and progress of the study. In addition, the Sponsor/Clinical coordinating center will maintain regular telephone and written communication with the EMS representatives and all Investigators.

The Sponsor will be given complete access to all components of the study facility that pertain to the conduct of this study and may be present to observe any aspect of the conduct of the study by medical and paramedical staff, including but not limited to drug preparations, dosing, sample collections, and clinical observations.

eCRFs will be monitored with sufficient frequency to assess the following: Subject randomization, compliance with protocol procedures, the completeness and accuracy of data entered into the eCRFs, verification of eCRF data against original source documents, and occurrence of AEs. Adequate time and all documents for these monitoring visits must be made available by the investigators. The clinical coordinating center, EMS representatives and investigators will permit trial-related monitoring, audits, REB review, and regulatory inspections, providing direct access to source data/documents.

11 Quality Control and Quality Assurance

Except for an emergency situation in which proper care for the protection, safety and well- being of the study subjects requires medical treatment, the study will be conducted as described in the approved protocol, 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 report. Protocol deviations will be reviewed regularly by the Sponsor to identify trends at study sites.

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 subjects. In addition, 100% of subjects enrolled are monitored for the presence of signed consent and Personal Information and Portable Electronic Documents Act (PIPEDA) documentation, where applicable.

Monitoring of the investigational sites will be conducted by the Sponsor or contracted to a qualified individual. 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. At site visits, the monitor will, as required, assess the progress of the study; check that the study data chosen for verification are authentic, accurate, and complete; verify that the safety and rights of subjects are being protected; compare original documents with data entered into the study database; and identify any issues and address their resolution.

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.

Additional on-site monitoring verification includes ongoing evaluation of the adequacy of site facilities and staff, site recruitment, subject randomization, the presence of regulatory documents, and specific review of documents and data. The initial performance-monitoring visit to a site takes place after the initial subject(s) are enrolled and will continue according to enrolment for the duration of the trial.

During the monitoring visit, any omissions and corrections to data submitted to the database will be noted and queries will be generated by the monitor and resolved by the site.

The close-out monitoring visit by the monitor will take place at the completion of subject enrollment and protocol required follow-up visits at the performance site. At that visit, the monitor will again review the presence of a regulatory file and verify documents for currency and completion. Sites will be instructed in the record retention of all trial documents. Principal Investigators are directed to close the trial and issue a final report to the REB. Finally, any additional special considerations for the auditing of any additional safety issues are made during this final monitoring visit.

11.1 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.

11.2 Protocol Amendments and Revisions

Should amendments and/or revisions to the protocol be required, they will be originated and documented by the Sponsor. All amendments and/or revisions will be made in compliance with Sponsor SOPs. All amendments will be submitted to the REB for approval prior to implementation.

It is the Sponsor’s responsibility to submit all revisions and amendments to regulatory authorities when necessary.

12 Ethics

12.1 Research Ethics Board/Institutional Review Board

This study will be conducted in substantial compliance with the principles and requirements of ICH GCP, Canadian Food and Drug Regulations, United States Code of Federal Regulations (including Title 21 Parts 50, 54, 56, and 312), the Declaration of Helsinki and the Canadian Tri-Council Policy Statement on Ethical Conduct for Research involving Humans (2), where applicable.

This protocol and the consent forms will be submitted to each hospital's and EMS Agency's REB. Before initiation of the study, a copy of the REBs' approval letters will be provided to the Sponsor and the membership list of the REB will be kept on file.

This protocol and the informed consent document and any subsequent modifications will be reviewed and approved by the REB responsible for oversight of the study. For subjects who cannot consent themselves, an LAR may sign the consent form. The consent form describes the purpose of the study, the procedures to be followed, and the risks and benefits of participation. A copy of the consent form must be given to the subject and/or the LAR; and this fact must be documented in the subject's record. It is at the discretion of the REB whether, if a consent is obtained from a LAR, and additional consent is needed when a subject had regained capacity.

SAEs will be reported to the REB according to their requirements.

13 Data Handling and Recordkeeping

13.1 Data Handling

The database used in the study will be a 21 CFR Part 11 compliant database. During the trial, clinical data reported in the eCRFs will be integrated into the clinical database under the responsibility of the Sponsor or their qualified representative. Quality control in the form of computerized logic and/or consistency checks will be systematically applied in order to detect errors or omissions.

In addition, 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.

After integration of all corrections in the complete set of data, the database will be released for statistical analysis.

13.2 Investigator Files/Retention of Documents

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) Subject Clinical Source Documents.

The Investigator's Study File will contain the Protocol/Amendments, CRFs, REB 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.

Subject clinical source documents (usually defined by the project in advance to record efficacy/safety parameters independent of the CRFs) would include subject hospital/clinic records, physician's and nurse's notes, appointment book, laboratory reports, ECG, X-ray, pathology and special assessment reports, signed consent forms, consultant letters, and source worksheets. 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 25 years. After that period of time the documents may be destroyed, subject 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.

13.3 Source Documents and Background Data

All Investigators shall supply the Sponsor, upon request, with any required background data from the study documentation or clinic records. This is particularly important when eCRFs are illegible or 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 subject confidentiality is protected.

13.4 Case Report Forms

For each subject randomized, an electronic CRF must be completed and signed by the Investigator. If a subject withdraws from the study, the reason must be noted on the CRF. All forms should be completed within five business days of subject visit.

All corrections will be tracked in the eCRF audit trail. The Investigator should ensure the accuracy, completeness, legibility, and timeliness of the data reported to the Sponsor in the CRFs and in all required reports.

13.5 Confidentiality

All imaging, evaluation forms, reports, and other records that leave the site are identified only by the site and subject number to maintain subject confidentiality. All records are kept in a locked file cabinet. Clinical information is not released without written permission of the subject, except as necessary for monitoring by REB, Health Canada, the Sponsor, or the Sponsor's designee.

All study Investigators at the clinical sites must ensure that the confidentiality of personal identity and all personal medical information of study subjects are maintained at all times. Federal legislation in Canada (PIPEDA), and provincial legislation where applicable, must be followed. On the CRFs and other study documents or image materials submitted, the subjects are identified only by study identification codes.

Personal medical information may be reviewed for the purpose of verifying data recorded in the CRF 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.

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STATISTICAL ANALYSIS PLAN

STATISTICAL ANALYSIS PLAN

NoNO, Inc.

Toronto, Ontario

Protocol NA-1-005

A MULTICENTER, RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED STUDY TO DETERMINE THE EFFICACY AND SAFETY OF INTRAVENOUS NA-1 INITIATED BY PARAMEDICS IN THE FIELD FOR ACUTE CEREBRAL ISCHEMIA WITHIN THREE HOURS OF SYMPTOM ONSET

(FRONTIER Trial)

28 March 2023

Version 3.0

FINAL

SIGNATURES OF APPROVAL

Jim Christenson, M.D. Coordinating Investigator Department of Emergency Medicine University of British Columbia Faculty of Medicine	Date
Michael Tymianski, M.D., Ph.D., President and CEO/Senior Medical Officer NoNO Inc.	Date
Redacted- personal information, M.S., Biostatistician II Redacted CRO name	Date
Redacted- personal information, M.S., VP, Biostatistics & Statistical Programming *Redacted CRO name*	Date

Version History

Version	Date	Change	Rationale
1.0 Draft	14 Nov 2014	Not Applicable	Original version
2.0	24 July 2019	Change in CRO	Change of CRO
		Augment Introduction and Trial Rationale	Update SAP with the impact of the ESCAPE-NA1 trial results
		Amend secondary and tertiary objectives	Update SAP to align with current protocol and change in objectives
		Augment information in SAP by adding details to - Endpoints - Study design - Schedule of Assessments - Randomization - Procedure for breaking randomization code - Protocol Deviations - Treatments - Pooling of Sites - Consent - Efficacy Outcomes and Analysis, including details of programming - Summary of Inferential Efficacy Analysis - Safety Analysis - Management of IDMC	Missing from previous SAP
		Added Confirmed Cerebral Ischemia Subset	New subset of interest
		Additional details managing Missing Data	Missing from previous SAP
3.0 Final	28 Mar 2023	Amend secondary and tertiary objectives	Update SAP to align with change in objectives
		Amend Background and Rationale	Update SAP with the impact of the ESCAPE-NA1 trial results
		Amend the following information in the SAP: - Study Design - Schedule of Assessments - Treatment-Emergent Adverse Events - Analysis Population Definitions - Missing Data Imputation	

		- Multiple Testing Procedure for Multiplicity - Primary Efficacy Analysis - Adverse Events - Vital Signs - Laboratory Results - 12-Lead Electrocardiogram Results - Prior and Concomitant Medications	
		Augment information in SAP by adding: - Table 2-1: Analysis Populations with Efficacy and Safety Outcome Measures - Study Progress Time	Missing From previous SAP

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LIST OF ABBREVIATIONS

AE	Adverse Event
AESI	Adverse Event of Special Interest
AIS	Acute Ischemic Stroke
ALDS	Academic Medical Center Linear Disability Score
ANCOVA	Analysis of Covariance
ASPECTS	Alberta Stroke Program Early CT Score
BI	Barthel Index
CACI	Confirmed Acute Cerebral Ischemia
CI	Confidence Interval
CRF	Case Report Form
CTA	Computerized Tomographic Angiography
eCRF	Electronic Case Report Form
ECG	Electrocardiogram
ED	Emergency Department
EMS	Emergency Medical Services
EQ-5D-5L	European Quality of Life, 5 Dimensions
EQ-VAS	EQ-5D-5L Visual Analog Scale
EVT	Endovascular Thrombectomy
FAST-MAG	Field Administration of Stroke Therapy - Magnesium
GCS	Glasgow Coma Score
ICH	Intracerebral Hemorrhage
IDMC	Independent Data Monitoring Committee
IRT	Item Response Theory
ITT	Intent-to-Treat
IV	Intravenous
LAMS	Los Angeles Motor Scale
LVO	Large Vessel Occlusion
MedDRA	Medical Dictionary for Regulatory Activities
mITT	Modified Intent-to-Treat
mRS	Modified Rankin Scale
n	Number of Observations
NIHSS	National Institutes of Health Stroke Scale
POM	proportional odds model
PP	Per Protocol
PSD-95	Post-synaptic density 95 protein
SAE	Serious Adverse Event
SD	Standard Deviation
SOC	System Organ Class
TEAE	Treatment-emergent Adverse Event
TIA	Transient Ischemic Attack
TICI	Thrombolysis In Cerebral Infarction
TEAESI	Treatment-emergent Adverse Event of Special Interest
tPA	Tissue Plasminogen Activator

1.0 INTRODUCTION

This document provides the details of statistical analyses planned for Protocol No. NA1-005. In addition, it discusses the statistical issues relevant to these analyses (e.g., sample data to be used and missing data), and it is informed by results obtained- from the completed phase 3 ESCAPE-NA1 trial[1] (summarized in Section 1.1.1.).

1.1 Background and Rationale

A stroke occurs when there is a blockage of an artery supplying blood flow to the brain (acute ischemic stroke; AIS), or bleeding into or around the brain (hemorrhagic stroke, or "ICH"). AIS should be treated as a medical emergency because 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. Many patients with a stroke either fail to reach hospital in time or are contraindicated for thrombolytic or mechanical reperfusion, the only available therapies for AIS. Of those who receive reperfusion therapy, only about 10% return to life as it was before. Thus, there is a compelling unmet need to develop neuroprotectants to lengthen the therapeutic window of reperfusion therapies, to enhance the outcomes of those receiving reperfusion therapies and to increase the proportion of patients who achieve functional independence following AIS.

Nerinetide, previously referred to as NA-1, is being developed to address this unmet medical need. It 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)[2]. PSD-95 is an abundant protein localized in post-synaptic densities of central nervous system neurons. 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.

FRONTIER trial aims to enroll AIS patients early in the prehospital setting, thereby initiating treatment at the earliest possible opportunity at which all or the majority of participants have salvageable brain. This strategy, enrolling participants criteria substantially similar to those of the FAST-MAG trial[3] provides an opportunity to target AIS patients early in their stroke progression. These patients may have the greatest amount of salvageable brain in order to benefit from neuroprotection, and to enhance further the impact of other therapies such as reperfusion therapies[4]. Our preclinical and clinical data support this notion.

The rationale for this study is as follows:

1. There is no convincing evidence from randomized controlled trials that neuroprotection can be of clinical benefit to patients with AIS. There is however, extensive preclinical evidence that neuroprotection is of greatest benefit for improving functional outcome in studies employing experimental animal stroke models in which the stroke is treated as early as possible.
2. The neuroprotectant, nerinetide, has been demonstrated to be highly effective in reducing stroke size and improving the functional outcome of experimental animals subjected to acute stroke, including rats and primates.[2,5]

3. Nerinetide has an excellent safety profile in preclinical animal studies, a human Phase 1 trial[6], and a human Phase 2 study (the ENACT trial),[7] Subjects in the ENACT trial were individuals who were being subjected to an endovascular procedure for intracranial aneurysm repair and to treatment with nerinetide or placebo.[1]
4. Nerinetide has shown promising results in reducing ischemic brain damage in humans having demographics similar to those of stroke patients[7]
5. There is a compelling need to develop neuroprotectants in order to increase the proportion of patients who achieve functional independence following AIS. Additionally, neuroprotectants may enhance the outcomes of those subjects who receive additional therapies such as pharmacological or endovascular recanalization or make more patients into candidates for endovascular or pharmacological recanalization treatment.

Nerinetide is being developed as an emergency drug aimed at reducing global disability in patients with acute cerebral ischemia if administered within three hours of symptom onset. The central aim of this study is to demonstrate the efficacy and safety of 2.60 mg/kg (up to a maximum of 270 mg) of nerinetide administered to patients with suspected acute stroke by paramedics in the field within three hours of symptom onset.

FRONTIER is anticipated to enroll patients with suspected stroke, based on pre-hospital stroke identification protocols, prior to hospital arrival. Upon arrival to the stroke center, they will undergo diagnostic studies anticipated to confirm a range of diagnoses including:

- Confirmed acute cerebral ischemia (AIS or TIA)
- Confirmed hemorrhagic stroke (ICH)
- Stroke mimicking conditions (e.g., hemiplegic migraine, brain tumor, post-ictal state)

Additionally, enrolled patients with confirmed cerebral ischemia will receive a range of in-hospital treatments as necessary, depending on their AIS/TIA characteristics, including treatment with reperfusion therapies comprised mainly of:

- Thrombolytics after nerinetide
- Endovascular thrombectomy (EVT) after nerinetide
- Thrombolytics + EVT after nerinetide

1.1.1 ESCAPE-NA1 Summary and Results

Since the launch of the FRONTIER trial, the phase 3 ESCAPE-NA1 trial[1] was completed. ESCAPE-NA1 (ClinicalTrials.gov Identifier: NCT02930018) was a multi-center, randomized, double blinded trial of nerinetide in subjects with AIS who were selected for endovascular thrombectomy (EVT). This was the first completed major trial of nerinetide in the AIS population, and the results provided important knowledge that informs the analyses of the FRONTIER trial.

In ESCAPE-NA1, patients with acute ischemic stroke due to large vessel occlusion were enrolled within a 12-hour treatment window, ASPECTS greater than 4 (Range 0-10; lower score suggests greater extent of acute ischemic changes) and vascular imaging showing moderate-to- good collateral filling as determined by multiphase CTA. They were randomized in a 1:1 ratio to receive

an intravenous infusion of nerinetide or saline. Randomization was stratified by prior intravenous alteplase treatment (tPA -yes/no) and declared initial endovascular device choice (stent retriever / aspiration device). All patients underwent endovascular thrombectomy and received alteplase if indicated. The primary outcome was a favorable functional outcome 90 days post-randomization, defined as a modified Rankin Scale (mRS) score 0-2. Secondary outcomes were measures of neurological disability, activities of daily living and mortality.

Among 1105 patients assigned to receive nerinetide (n=549) or saline (n=556), 61.4% in the nerinetide group and 59.2% in the saline placebo group achieved a mRS 0-2 at 90 days (adj odds ratio (OR)= 1.14; 95% confidence interval [CI_{95}], 0.87-1.51; p=0.333).

Separate analyses of each of the fully randomized strata of subjects who did (n = 659) and did not (n = 446) receive alteplase prior to nerinetide were conducted. These showed no efficacy of nerinetide in the stratum that received prior alteplase, consistent with the cleavage and inactivation of nerinetide by plasmin, the product of prior alteplase treatment[9].

By contrast, in the no-alteplase stratum, subjects treated with nerinetide showed improved functional independence, reduced mortality and reduced infarction volumes. For the no-alteplase stratum, the relative treatment effect of nerinetide for an mRS 0-2 was 19.3% [OR 1.657, CI_{95} 1.055-2.603, $p_{(nominal)}$=0.028], the relative difference for mortality was 39.6% [OR 0.572, CI_{95} 0.323-1.013; $p_{(nominal)}$=0.055] and the relative reduction of mean infarct volume was 22% [26.74 ml in nerinetide vs. 39.21 ml in placebo; LS mean difference (cubic root transformation) -0.33, CI_{95} -0.662, -0.003; $p_{(nominal)}$=0.048]. Moreover, nerinetide reduced the number of subjects with worsening of their index stroke either due to increase of the ischemic territory (2.7% in nerinetide vs. 5.3% in placebo) or hemorrhagic transformation (0.5% in nerinetide vs. 2.2% in placebo). Nerinetide had an acceptable safety profile in both the alteplase and no alteplase strata.

Importantly, in a combined analysis of patients from the ESCAPE[10] and ESCAPE-NA1[1] trials that failed to undergo successful reperfusion therapy, outcomes were similar to those that received only best medical therapy for their stroke[11]. This underscores the criticality of reperfusion when a stroke is caused by a large vessel occlusion (LVO). Consistent with this, in an as-yet unpublished but pre-specified analysis in ESCAPE-NA1, nerinetide in the no-alteplase stratum was more effective in LVO patients who experienced adequate reperfusion (TICI 2b-3), and less effective in the absence of reperfusion (TICI 0-2a). This important finding may have profound implications to the efficacy of nerinetide in the FRONTIER trial, and is therefore taken into account in this analysis plan.

Also importantly, the absence of treatment benefit of nerinetide in the 659 subjects in the alteplase stratum was likely due to cleavage of nerinetide by plasmin, activated by prior treatment with alteplase[9]. This resulted in reduced plasma nerinetide levels, confirmed by PK sampling in a subset of trial subjects, to levels that likely were subtherapeutic. In brief, the effect of nerinetide is nullified when its plasma Cmax is reduced to approximately 5µg/ml. The PK/PD relationships observed in the ESCAPE-NA1 alteplase stratum are consistent with those observed in our preclinical rat and primate studies9. This important finding also has profound implications to the FRONTIER trial because in FRONTIER, unlike in ESCAPE-NA1, all subjects who receive alteplase receive it after, and not before, nerinetide. In this scenario, nerinetide is administered in

the absence of circulating plasmin and should not be cleaved. Our preclinical PK data in primates suggest that separating nerinetide and alteplase by as little as 10 minutes preserves the PK of nerinetide. Our other studies in rats indicated that giving nerinetide 30 minutes before alteplase was highly neuroprotective, and was potentially synergistic with alteplase. FRONTIER is an opportunity to explore this clinically.

In summary, the ESCAPE-NA1 results provided support for clinical efficacy among the large (446 subjects), pre-specified, randomized and scientifically non-arbitrary stratum of AIS patients not receiving alteplase, and provided important knowledge that should be factored into the analysis of the FRONTIER trial.

1.1.2 Implications of ESCAPE-NA1 Findings for FRONTIER

The knowledge gained from the separate analyses of the alteplase and no-alteplase fully-randomized strata of the ESCAPE-NA1 trial has the following important implications for FRONTIER:

1. In addition to improving functional independence, nerinetide may slow the progression of stroke, and/or reduce mortality – two additional orthogonal endpoints of clinical relevance.
2. ESCAPE-NA1 has confirmed two important potentially effect-modifying interactions that have profound implications for FRONTIER:
    A. The effectiveness of nerinetide may be reduced in the absence of adequate reperfusion.
    B. The effectiveness of nerinetide is nullified by the prior or concurrent administration of alteplase.

Since thrombolysis with alteplase is only undertaken after hospital arrival, and FRONTIER subjects receive study drug prior to hospital arrival, this study provides a unique opportunity to evaluate the effectiveness of nerinetide without the effect modification caused by nerinetide cleavage due to prior alteplase treatment.

Incorporating knowledge learned from ESCAPE-NA1, including of the potential effect-modifying interactions of reperfusion and alteplase is key to extracting meaning from FRONTIER. This has already been done in the Phase 3 ESCAPE-NEXT trial (clinicaltrials.gov NCT04462536) which is aimed at confirming the encouraging results in the no-alteplase stratum of ESCAPE-NA1 of subjects with AIS who receive reperfusion with EVT. This SAP addressed similar issues, which are central to NoNO's development plan for nerinetide, by addressing and adjusting the effect modification of reperfusion in its planned analysis.

1.2 Objectives

1.2.1 Primary Objectives

The primary objective is to determine the efficacy of nerinetide in reducing global disability in patients with acute stroke.

1.2.2 Secondary Objectives

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

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

*Worsening of stroke is defined as progression, or hemorrhagic transformation, of the index stroke as documented in the study CRF that (i) is deemed life-threatening and/or (ii) results in increased disability as gauged by a ≥4 point increase from lowest NIHSS during hospitalization and/or (iii) results in death.

1.2.3 Tertiary Objectives

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

- Improving functional independence
- Improving health-related quality of life.
- Increasing the proportion of subjects whose symptoms fully return to baseline function within 24 hours of symptom onset.
- Increasing the proportion of subjects who receive reperfusion therapy.
- Increasing the proportion of subjects who receive thrombolysis.
- Reducing functional dependence at Day 30
- Reducing physical disability.

1.2.4 Leading Safety Objectives

The leading safety objectives are to determine the effect of administering a target dose of 2.60 mg/kg (up to a maximum dose of 270 mg) IV infusion of nerinetide within three hours of symptom onset by paramedics in the field on SAEs and on 90-day mortality.

1.3 Endpoints

1.3.1 Primary Efficacy Endpoint

The primary efficacy endpoint is the percentage of responders, using a sliding dichotomy on the mRS scale at Day 90.

1.3.2 Secondary Efficacy Endpoints

The secondary efficacy endpoints are:

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

2. A reduction in mortality as defined by event rate (proportion, expressed as a percentage) for mortality over the 90-day study period.
3. Proportion of subjects with worsening of stroke* over the 90-day study period.
4. Proportion of subjects with good neurological outcome, as defined by a score of 0-1 on the NIHSS at Day 90 or the last rating.
5. Proportion of subjects with functional independence in activities of daily living, as defined by a score of ≥ 95 on the Barthel Index at Day 90 or the last rating.

*Worsening of stroke is defined as progression, or hemorrhagic transformation, of the index stroke as documented in the study CRF that (i) is deemed life-threatening and/or (ii) results in increased disability as gauged by a ≥4 point increase from lowest NIHSS during hospitalization and/or (iii) results in death.

1.3.3 Tertiary Efficacy Endpoints

The tertiary efficacy endpoints include:

1. Proportion of subjects with functional independence, as defined by a score of a) 0-2 and b) [1] 0-1 on the mRS at Day 90 or the last rating.
2. Health-related quality of life, as measured by the EQ-5D-5L at Day 90 or the last rating[1].
3. Proportion of subjects with acute cerebral ischemia whose symptoms fully return to baseline function within 24 hours of symptom onset[1].
4. Proportion of subjects who receive any type of reperfusion therapy[1].
5. Proportion of subjects who receive thrombolysis[1].
6. Favorable outcome at Day 30 or last rating prior to Day 30, as described for the primary endpoint[1].
7. Physical disability, as measured by the ALDS at Day 90[1].

[1]Analysis to be conducted only if efficacy outcomes warrant further exploration

1.3.4 Leading Safety Endpoints

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

1.4 Study Design

This study is a multicenter, randomized, double-blind, placebo-controlled, single dose efficacy and safety study of nerinetide initiated prehospital in the ambulance. Enrolled subjects will be given a single, intravenous, target dose of 2.6 mg/kg (up to a maximum of 270 mg) of nerinetide or placebo.

A total of 558 male and female subjects between the ages of 40-95 years with suspected acute stroke will be identified in the field by licensed, trained paramedics using the approved stroke protocol in use by the local EMS system. Stroke severity will be graded by the Los Angeles Motor Scale (LAMS). Subjects will be approved for the study by an on-call trial physician by cellular phone.

The paramedics will then begin study drug administration. Randomization is defined as the moment a subject receives any amount of study drug. Upon arrival at the emergency department (ED), subjects will receive standard-of-care treatment, which may include thrombolytics and/or endovascular thrombectomy, as appropriate.

All subjects will be followed for 90 days (or until death if prior to 90 days). The end of 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-person visit is not possible the participant can be contacted by telemedicine (preferred) or by telephone (last option).

The Schedule of Assessments is presented in Table 1-1.

Table 1-1: Schedule of Assessments

Procedures	Baseline Day 1 (Pre-ED Arrival)	Day 1 ED Arrival	Day 2 (24 Hours ±6 Hours) in Person	Day 4 (±1 Day) in Person	Day 30[9] (±7 Days)	Day 90[9] (±30 Days)
In Ambulance						
Brief Medical and Surgical History	X					
Brief Demographics (age, sex, major vascular risk)	X					
Local approved stroke protocol, LAMS, inclusion and exclusion criteria, GCS	X					
Blood Pressure, Heart Rate	X[1]					
Weight	X[2]			X[3]		
Initiate Study Drug Infusion	X					
In Hospital						
Prior Medications (within 3 days)		X				
Complete Medical History		X	X			
Blood Pressure, Heart Rate		X[4]	X	X		
Temperature, SaO_2		X[4]	X			
CBC, electrolytes, creatinine, glucose, INR, PTT[5]		X	X			
Pregnancy Testing[6]		X				
12-lead ECG[5]		X				
Serious Adverse Events (including symptomatic hemorrhagic transformation and recurrent ischemic stroke)	Collected from Day 0 to Day 90/end of study					
Adverse Events	Collected from Day 0 to Day 30					
Concomitant Medications	Collected from Day 0 to Day 4					
NIHSS		X[7]	X	X		X
mRS			X[8]	X	X	X
Assess whether symptoms have resolved within 24 hours of symptom onset			X			
Barthel Index				X	X	X
EQ-5D-5L					X	X
ALDS						X

[1] Blood pressure to be recorded on eligibility determination, within 10 ±5 minutes or less prior to the start of study drug infusion, and immediately upon (but no later than 15 min after) completion of study drug infusion.
[2] Determined by paramedic by first asking the patient, secondly asking a family member or third, by paramedic estimation. This weight will be used for calculating the volume of study drug to be administered.
[3] Actual weight measured in hospital within 4 days. If actual weight cannot be measured due to, for example severe illness, determine weight by first asking the subject, second asking by asking a family member or third by estimation.
[4] Per standard-of-care. The assessment of vital signs closest to the 20- min time-point after ED arrival will be entered into the eCRF.
[5] Testing per standard-of-care, sample to be reported in CRF is the one closest to visit window.
[6] For women of child-bearing potential only; per standard-of-care.
[7] No more than 4 hours post-dose; per standard-of-care.
[8] Premorbid mRS status and mRS status at acute stroke hospital discharge.
[9] At Day 30 and Day 90 it is preferred that subjects will return to clinic. If a in clinic visit is not possible the subject can be contacted by telemedicine (preferred) or by telephone (last option).

1.5 Sample Size Determination

The primary outcome variable is the overall proportion of subjects experiencing a favorable functional outcome 90 days post randomization. Sample size projections assume, based on the Field Administration of Stroke Therapy – Magnesium (FAST-MAG) experience[3], that approximately 72% of randomized subjects will have AIS, 24% will have intracerebral hemorrhage (ICH) as their stroke subtype, 4% will have stroke-mimicking conditions, and that treatment benefit is obtained mainly in patients with acute cerebral ischemia. Assuming a 26% overall responder rate for the placebo group (as reported in the FAST-MAG trial, J. Saver, personal communication, 2013) using the sliding dichotomy definition of responder (Table 7-1), there will be an estimated 80% power to detect a 12% absolute effect difference between response rate (proportion of responders) with nerinetide and placebo, at alpha level 0.05, 2-sided with a planned sample size of 506 evaluable subjects, randomized 1:1, 253 per group. The 12% absolute response rate difference is judged to be the minimally clinically important difference to justify prehospital administration of nerinetide; it was selected in part because it is similar in magnitude to a previously proven clinically important difference: The absolute response rate to tissue plasminogen activator (tPA)[12]. The sample size will be inflated 10% to account for loss-to-follow-up and drop-outs (n=558, 279 per group). If loss-to-follow-up and drop-outs exceeds 10% by the time that original enrollment reaches approximately 80%, the sample size may be inflated an additional 5% (n = 586, 293 per group).

1.6 Randomization

Randomization is by pre-specified permuted block design, allocating nerinetide or placebo in a 1:1 ratio, and is stratified by EMS hub. For the purpose of clarity, an EMS hub is defined according to the EMS system covering a given geographic region, not the ambulance depot receiving and storing boxes of drug vials. In this scenario, a single EMS hub may have one or more ambulance depots. Randomization codes will be generated at the manufacturer level employing a computerized random number sequence and boxed by block in ascending numerical order. Ambulance depots of EMS hubs will receive single boxes of 42 vials containing a whole number of pre-specified permuted blocks of concealed size (e.g., seven permuted blocks of six vials arranged in a 1:1 ratio of nerinetide : placebo). In order to maintain the randomization sequence at the ambulance depot, trained study personnel will assign the study drug vials to ambulances in ascending numerical order based on the clear markings on the vial and the box. Personnel will also assign all 42 vials from one box before opening the next. A single study drug vial will be assigned and tracked into the ambulance and placed in the on-board miniature refrigerator per study working practices. Drug accountability, distribution and tracking for each ambulance depot will be documented and available for monitoring purposes.

The time of randomization is defined as the moment a subject receives any amount of study drug. The moment of randomization will be rigorously documented. All patients that are randomized will be accounted for in the trial database. The paramedic will record the randomization time, which will be entered into the electronic case report form (CRF) and ambulance call report, respectively. The paramedic will record both the vial number and time of randomization after each randomization occurs to ensure an accurate determination of all subjects

randomized and exposed to the study drug. Each study drug vial will be individually labeled with a unique identification number by the packaging company to preserve blinding. This unique identification number will also serve as the subject's randomization number. Once a subject is randomized, re-stocking participating ambulances with the next study drug vial in that EMS hub's randomization sequence will be done per the local EMS hub working practice.

1.7 Blinding

The study is conducted in a blinded manner. All subjects, paramedics, investigators, their clinical staff, the clinical coordinating center, the data management group, independent adjudication committee, local hospital laboratories and the sponsor staff and delegates will be blinded to the randomization codes. The IDMC will be unblinded to safety data to ensure a detailed analysis of safety. In order to ensure confidentiality and to minimize bias, the safety information will be provided to the IDMC by a group that is independent of the sponsor and blinded project team implementing the trial. A firewall will be maintained between the IDMC (unblinded) and the project staff (blinded). The IDMC will remain blinded to efficacy data throughout the trial unless significant concerns about safety develop.

The IDMC reports and analyses for Closed Sessions will be organized by treatment arm ("unblinded"). In order to ensure confidentiality and minimize bias, the information will be provided to the IDMC by a group that is independent of the sponsor and blinded project team implementing the trial. A firewall will be maintained between the IDMC (unblinded) and the project staff (blinded).

There will be two primary statisticians involved in the trial. The project statistician, who remains blinded to treatment assignment throughout the trial until final analysis after database lock, will be responsible for interacting with the trial management committee and project team and will conduct the final analyses for the clinical study report. He/she is also responsible for finalizing this Statistical Analysis Plan, which includes decisions about the final analysis populations and changes to the primary and other analyses, prior to the final database lock and unblinding. The second statistician, the independent reporting statistician to the IDMC, is a member of the unblinded Independent Statistical Center, which runs the safety reports and analyses for the IDMC. The independent reporting statistician will present the unblinded reports to the IDMC.

The person responsible for generating the randomization codes and the study drug packaging company will also be unblinded.

Randomization data will be kept strictly confidential, accessible only to authorized persons, until the time of unblinding.

Only when the study is completed and the database is locked will the investigators, clinical staff, and the trial sponsor and its staff be unblinded.

1.7.1 Procedure for Breaking the Randomization Code

In the event of an emergency and following a discussion with the Medical Monitor, the randomization code for an individual subject may be revealed to the site Investigator. The randomization code would then be obtained by phone or e-mail from the person responsible for

generating the randomization code, following authorization by the Medical Monitor. Any case that is unblinded in this way will be documented in central files.

The unblinded person will provide only to the site Investigator by email the patient dose allocation information as well as the date of unblinding, site number, Investigator name and subject number. Any case that is unblinded in this way will be documented in central files. Only the Investigator requesting the unblinding will receive the unblinding information. It is not expected that there is any clinical instance where unblinding will be required.

Health authorities may request a code-break in the case of an SAE as described in International Conference on Harmonization E2A. In this case, the code will be broken only for the subject(s) in question. The information resulting from a code-break will not be communicated to the Sponsor or Investigator. This code will be broken by the person responsible for generating the randomization code and communicated directly to the legally designated third party.

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

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 the start of study drug administration.

Prior and Concomitant Medications: Prior medications are defined as those taken within three days of treatment initiation. Concomitant medications are defined as those taken after the start of study drug administration through and including the Day 4 visit. All prior and all concomitant medications will be recorded on the electronic case report form (CRF).

Randomization: Randomization is deemed to occur if a subject receives any amount of study drug.

Treatment-Emergent Adverse Event: A treatment-emergent adverse event (TEAE) is one which first occurs or worsens in severity or frequency after study drug has been administered through and including the Day 90 visit. AEs are collected through the Day 30 visit and SAEs are collected through the Day 90 visit/last study visit. TEAE is defined as an AE that first occurs or worsens in severity after study drug has been administered through to and including Study Day 30 when non-serious and through to and including Study Day 90 when serious. Those AEs/SAEs that start at the same time and date as the study drug administration and those that first occur or worsen in severity after the start of study drug administration will be considered TEAEs.

AEs reported with partial or missing dates will be considered to be TEAEs.

Primary Efficacy Analysis Population: This is a defined by the stepwise process described in Section 7.1.3.1.

Reperfusion Therapy: Defined as the use of endovascular thrombectomy, thrombolysis, or both, within 24 hours of hospital arrival.

2.0 ANALYSIS POPULATION DEFINITIONS

FRONTIER subjects consist of a number of important analysis populations for primary, secondary, or exploratory analyses. These are defined below.

2.1 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.

2.2 Modified Intent-to-Treat Population

The modified intent-to-treat (mITT) population is defined as all subjects receiving any amount of study drug and with at least one post-dose Modified Rankin Scale (mRS) assessment. Each subject will be analyzed according to the treatment group to which he/she was randomly assigned. Subjects who do not receive any amount of study drug are not considered to be randomized.

2.2.1 Rationale for Modified Intent-to-Treat Population

Randomization for the purposes of this unique trial design is defined as the moment a subject receives any amount of study drug. The moment of randomization will be rigorously controlled. All patients that are randomized will be accounted for in the trial database. The on-call trial physician will record the randomization number and date and time will be recorded after each randomization occurs to ensure an accurate determination of all subjects randomized and exposed to the study drug.

However, as the primary endpoint is reduction in global disability based on the mRS assessment, a post dose assessment is necessary. Thus, the only difference between mITT and ITT in this trial is that mITT analysis will be conducted on those subjects for which there is at least one post-dose mRS assessment..

2.3 Primary Efficacy Analysis Population

This is a defined by the stepwise process described in Section 7.1.3.1. This process ensures that the analysis plan is aligned with knowledge gained from the ESCAPE-NA1 trial about the effect modifiers discussed in Section 1.1.2.

2.4 Per-Protocol Population

The primary analysis will be repeated on the Per-Protocol population. The PP population comprises all subjects in the Primary Efficacy Analysis population 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 (see Section 6.2 for further details). This analysis population will be defined via a blinded review of the study data prior to final database lock and study 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). .

2.5 Confirmed Acute Cerebral Ischemia Population

The 'Confirmed Acute Cerebral Ischemia (CACI) population' will be defined as subjects in the mITT population who are deemed by the treating stroke physician as having sustained a diagnosis of acute ischemic stroke of any severity or a transient ischemic attack. Subjects who are diagnosed with a hemorrhagic stroke or with an alternate diagnosis of a stroke-mimicking condition including but not limited to brain tumor, seizure, hemiplegic migraine or conversion reaction will not be deemed to have acute cerebral ischemia and will not be included in this subset. This analysis will be performed in a similar manner to the primary analysis.

2.6 Subjects Treated with a Reperfusion Therapy Population

The 'subjects treated with a Reperfusion Therapy analysis population' will be defined as those subjects in the mITT population who were documented in the CRF as having received thrombolytics, EVT, or both within 24 hours of hospital arrival.

2.7 Subjects Treated with Thrombolytics Population

The 'subjects treated with thrombolytics population' will be defined as those subjects in the mITT population who were documented in the CRF as "Was IV tPA given for this presenting stroke event- Yes" within 24 hours of hospital arrival.

2.8 Subjects Treated with Endovascular Thrombectomy

The 'subjects treated with endovascular thrombectomy population' (EVT) will be defined as those subjects in the mITT population who were documented in the CRF as "Was endovascular intervention performed- Yes" within 24 hours of hospital arrival.

2.9 Intracerebral Hemorrhage (ICH) Population

The 'Confirmed ICH analysis population' will be defined as those subjects in the mITT population who were documented in the CRF as having a discharge diagnosis of "intracerebral hemorrhage".

Table 2-1: Analysis Populations with Efficacy and Safety Outcome Measures

	Safety	Per Protocol[1]	mITT[1]	Confirmed acute cerebral ischemia (CACI)	Subjects with reperfusion therapy			Subjects without reperfusion therapy[2]			
					Any reperfusion therapy[1]	Treated with EVT[2]	Treated with thrombolytics	All Subjects without reperfusion therapy	CACI without reperfusion therapy	Confirmed ICH	Other
Primary Efficacy Outcomes											
mRS Responder		X	X	X	X	X[2]	X	X[2]	X[2]	X[2]	X[2]
Secondary Efficacy Outcomes											
mRS Shift			X	X	X	X[2]	X	X[2]	X[2]	X[2]	X[2]
Mortality			X	X	X	X[2]	X	X[2]	X[2]	X[2]	X[2]
Worsening of Stroke			X	X	X	X[2]	X	X[2]	X[2]	X[2]	X[2]
NIHSS (0-1 vs >1)			X	X	X	X[2]	X	X[2]	X[2]	X[2]	X[2]
Barthel Index			X	X	X	X[2]	X	X[2]	X[2]	X[2]	X[2]
Tertiary Efficacy Outcomes											
mRS 0-2 and 0-1[2]			X		X						
EQ-5D-5L			X[2]		X[2]						
Return to baseline within 24 hours			X[2]		X[2]						
% Received Reperfusion Therapy			X[2]		X[2]						
% Received Thrombolysis			X[2]		X[2]						
mRS Responder at Day 30			X[2]		X[2]						
ALDs[2]			X[2]		X[2]						
Safety											
# SAEs	X-All			X	X[2]					X	
Mortality	X-All			X	X[2]					X[2]	

[1]Analysis will be done for the primary efficacy analysis population for efficacy as determined by the interaction effect and proportionality tests per Section 7.1.3.1.
[2]Analysis to be conducted only if efficacy outcomes warrant further exploration

3.0 INTERIM EFFICACY ANALYSES

There is no planned interim analysis for efficacy this study.

4.0 DATA REVIEW

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

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

5.0 MISSING DATA AND DATA TRANSFORMATION

5.1 Missing Data

Every effort will be made to keep missing data, particularly the Day 90 outcome assessments, to a minimum. However, some missing data may be inevitable due to the deferred consent process (i.e. subjects may refuse to continue), withdrawal of consent, or loss to follow-up. Deceased subjects will be assigned scores of 6 on the mRS, 42 on the NIHSS, and 0 on the Barthel Index and will be counted as non-responders. Irrespective of the Primary Efficacy Analysis Population, all imputations for missing data will be conducted on the mITT population.

5.1.1 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 subject 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 subject 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 subject 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 subject 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% of subjects in the mITT population are missing the Day 90 mRS score (and hence use some other post-dose mRS value for the primary analysis), four sensitivity analyses will be performed on the primary endpoint: (1) multiple imputation (2) worst score imputation and (3) best score imputation and (4) two-way tipping point analysis. Given that the mITT population excludes randomized participants who do not have at least one post-dose mRS, the same sensitivity analyses will be repeated on all participants who received any amount of study drug in the study, and in the primary efficacy analysis population.

(1) Multiple Imputation

Under the assumption that the missing data are missing at random, subjects with missing mRS results at the Day 90 time point will have these individual mRS results imputed.

Step 1: Imputation

For each missing mRS result, 50 imputations will be made using SAS PROC MI on the unimputed mRS data with treatment, timepoint, and EMS hub in the BY statement and mRS in the VAR statement. The number of multiple imputations will be 50, leading to 50 completed datasets.

Step 2: Analysis of completed datasets

The imputed data for the primary endpoint will be analyzed using the same method as originally used for the primary efficacy analysis. This will lead to 50 estimates for the proportion of response within each treatment group.

Step 3: Inference

The estimates will be averaged, and the associated standard errors will be summarized based on within-imputation and between-imputation variance, as is customarily done in multiple imputation. PROC MIANALYZE will be used to summarize the 50 estimates, yielding a final estimate with associated 95% CI.

(2) Worst Score Imputation

For this sensitivity analysis, all missing Day 90 mRS values in the mITT population will be assigned a value of 6, which is the worst score on the mRS scale.

(3) Best Score Imputation

For this sensitivity analysis, all missing Day 90 mRS values in the mITT population will be assigned a value of 0, which is the best score on the mRS scale.

(4) Two-Way Tipping Point Analysis

A two-way tipping point analysis will be used as a sensitivity analysis for the primary analysis in order to determine whether the results of the primary analysis remain robust in the event that missing data are not missing at random.

5.1.2 Imputation of Mortality

For the analysis of rate of mortality, for subjects 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 subject will be imputed as Dead at Day 90.

5.1.3 Imputation of NIHSS data

Deceased subjects will be assigned a score of 42 on the NIHSS and be counted as non-responders. Missing NIHSS at Day 90 will be imputed using the median score obtained at Day 90 in the trial.

5.1.4 Imputation of BI data and EQ-5D-5L

Deceased subjects will be assigned a scores of 0 on the Barthel Index (BI) and be counted as non-responders, and counted as non-responders on the EQ-5D-5L.

Missing BI and EQ-5D-5L data at Day 90 will be imputed using the last observation carried forward (LOCF) imputation as follows:

LOCF: Subjects who are missing 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 data will be imputed to the median score obtained at Day 90 in the trial.

5.2 Data Transformation

No transformation of the data is planned.

6.0 STATISTICAL METHODS

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

Unless otherwise noted, categorical data will be summarized for each treatment group using counts and percentages, with the denominator for percentages being the number of subjects in the population of interest. Percentages are rounded to one decimal place. 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 may be reported as the median, interquartile range, 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, and p-values. Unless stated otherwise, all statistical tests will be 2-sided hypothesis tests performed at the 0.05 level of significance. A sequential fixed multiple testing procedure will be used to protect the overall trial false positive rate, and is described in Section 6.6.

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

6.1 Subject Disposition and Demographics

Subject disposition will be summarized and tabulated separately for all subjects in the primary efficacy population, mITT, 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.

6.2 Protocol Deviations

The number and percentage of Subjects in the mITT population with major protocol deviations, as listed below, will be summarized by treatment group and overall. Major protocol deviations will be reviewed per subject prior to database lock and may result in the subject being removed from the PP analysis. These include:

- Enrollment did not comply with inclusion criteria
- Enrollment did not comply with exclusion criteria

- Study Drug Dosing- subjects who did not receive a complete dose and/or vial warmed and/or received the study drug infusion >15minutes.
- Other major protocol deviation as determined by the Sponsor.

6.3 Treatments

As per the protocol, each participating ambulance will be stocked with one single use vial of nerinetide or matching placebo. Provided that adequate IV access has been obtained, there are no issues with the study drug vial and the on-call trial physician has authorized study drug preparation, the paramedic will enroll the subject. The time of randomization is defined as the moment a subject receives any amount of study drug. Weight based dosing is conducted based on the subject's estimated weight. A table is provided to the paramedic, in order to establish the volume of study drug to be withdrawn from the drug vial and injected into a 50 ml bag of saline, which is then mixed and infused over 10+/-1 minute through an infusion pump.

Table 6-1: Weight Based Dosing

Estimated Weight (kg)		Estimated Weight (lbs)		Syringe Volume (mL) to Transfer to
Low	High	Low	High	50 mL Saline IV Mini-bag
≥45	≤49	≥ 99	≤108	6
>49	≤54	>108	≤119	7
>54	≤59	>119	≤130	7
>59	≤64	>130	≤141	8
>64	≤69	>141	≤152	9
>69	≤74	>152	≤163	9
>74	≤79	>163	≤174	10
>79	≤84	>174	≤185	11
>84	≤89	>185	≤196	11
>89	≤94	>196	≤207	12
>94	≤99	>207	≤218	13
>99	≤120	>218	≤264	13.5

Under this approach, an IV solution of 20mg nerinetide /ml will be given to subjects with a body weight < 105 kg to achieve a final target dose of 2.6 mg nerinetide/kg. subjects weighing >99 and ≤120 kg will each receive a total dose of 270 mg of study drug (i.e., the entire 13.5 ml contained in the study drug vial). However, per-protocol, at the time of dosing, the subject's weight is estimated by the paramedics. A second weight will be reported in the hospital. Discrepancies between the paramedic weight estimate and hospital weight may result in some subjects weighing >105 kg receiving less than 270 mg, and some subjects weighing < 105 kg receiving the full 270 mg dose. The following measures of the timeliness of the interventions and of exposure will be computed as follows (Summary of Exposure and Compliance).

1. Number of subjects who received any amount of nerinetide
2. Number of subjects with early study drug cessation.
3. Descriptive statistics for:

- Duration of study drug infusion (minutes)
- Duration of study drug infusion (categorical): < 9 minutes, 9-11 minutes, > 11 minutes
- Dosing compliance (%) = 100*[A+B]/C , where

  A = number of subjects who received 2.6 mg nerinetide/kg body wt;

  B = number of subjects who received 270mg total dose;

  C = number of subjects who received any amount of nerinetide. As dosing compliance (per protocol) is the subject of this calculation, "kg body wt" in this instance will be the weight (paramedic or hospital) obtained at the time of dosing.

- Actual exposure (mg nerinetide/kg body wt.) = [(20mg nerinetide/ml of IV solution)*ml dose received]/kg body wt., where "kg body weight" was obtained in-hospital.
- Relative exposure (%) = (Actual exposure*2.6 mg nerinetide/kg body wt.) *100 where "kg body weight" was obtained in-hospital.

All measures will be summarized for the mITT, population. Individual exposures will be listed by treatment arm and subject.

6.4 Study Progress Time

The following treatment workflow parameters and hospitalization duration parameters will be summarized by treatment group and overall on mITT Population.

- Time from stroke symptom onset to start of infusion
- Time from infusion start to ED arrival
- Time from infusion start to thrombolytics start, if applicable
- Time from infusion start to endovascular thrombectomy start, if applicable.
- Discharge Destination
- Patient Disposition (Day 90/End of study)

6.5 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 as determined by paramedics (in kg), and weight as determined in hospital (in kg). Baseline characteristics include, but are not limited to: vascular risk factors, side of stroke, type of stroke (ischemic, hemorrhagic, TIA or Stroke mimic (Other/undetermined)), baseline LAMS score, baseline Glasgow Coma Score, time since last seen normal, whether the subject received thrombolytics within 24 hours of hospital arrival and whether the subject received endovascular thrombectomy within 24 hours of hospital arrival. The summaries will be provided for the primary efficacy population, mITT, Per-Protocol, and Safety populations. Inferential statistics (i.e., p-values or CI) will not be provided for these data.

Past stroke focused medical history will be summarized. All medical history will be listed.

6.6 Pooling of Sites (EMS Hubs)

If there is an EMS hub with fewer than nine randomized subjects in the mITT population, that EMS hub will be pooled with the EMS hub with the next smallest number of subjects in the mITT population. If this results in a pooled grouping still having fewer than nine subjects, then this pool will be combined with the EMS hub with the next smallest number of subjects in the mITT population and so on until the pooled grouping has nine or more subjects.

6.7 Multiple Testing Procedure for Multiplicity

All tests will be conducted with two-sided level of significance alpha = 0.05. A fixed sequence multiple testing procedure will control the overall experiment-wise error rate for the trial. 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 below, after the first test which fails. All tests that follow the first failed test are considered exploratory. For the purpose of clarity, since the first secondary analysis of the ordinal mRS scores will employ a proportional odds model (POM), if the proportional odds assumption fails, this secondary analysis will not be performed, and the remaining secondary tests will still be considered to be protected. There is no planned interim futility or overwhelming efficacy analysis.

The fixed sequential order for testing in the **primary efficacy analysis population** is:

1. Primary efficacy endpoint
2. Secondary efficacy endpoints, as specified in the order presented below:
    1. Shift to reduced functional dependence analyzed across the whole distribution of scores on the mRS at Day 90 or the last rating.
    2. Mortality rate (proportion, expressed as a percentage) for mortality over the 90-day study period.
    3. Proportion of subjects with worsening of stroke over the 90-day study period
    4. Proportion of subjects with good neurological outcome, as defined by a score of 0-1 on the NIHSS at Day 90 or the last rating.
    5. Proportion of subjects with functional independence in activities of daily living, as defined by a score of ≥ 95 vs 0-90 on the Barthel Index at Day 90 or the last rating.

The remaining efficacy analyses described in this SAP are exploratory and will not be alpha-protected.

6.8 Consent

The study employs an exception to consent approach to enrollment so as not to interfere with the urgent medical needs of patients with suspected acute stroke. Subjects are then informed of the

study after arrival at the ED and consent is sought for the remaining follow-up from the subject once they regain capacity or a legally authorized representative becomes available. Any information obtained when the researchers were acting on an exception to consent will remain part of the study information.

Since subjects in this protocol are enrolled under an exception to consent approach, when a subject or legally authorized representative declines further participation this will be deemed a 'decline of further participation' whereas when a subject or legally authorized representative consent and subsequently declines, this will be deemed to be a withdrawal of consent. The number of subjects who withdraw consent or decline further participation prior to the evaluation of the 90-day outcomes will be listed by treatment arm for the safety population. A Fisher's exact test will evaluate whether there were differences in consent withdrawals between treatment arms in each analysis.

7.0 EFFICACY ANALYSIS

The analysis populations and corresponding efficacy analyses are summarized in Table 2-1.

Summary statistics will be presented. For continuous endpoints, the summary statistics will generally include: number of subjects with data, mean, standard deviation, median, quartiles, and range. For categorical endpoints, the summary statistics will generally include: number of subjects in corresponding analysis population, number and percentage of subjects in each category.

7.1 Primary Outcome Variable Analysis

7.1.1 Primary Outcome

The primary endpoint used in this trial will be global disability as measured by the mRS at Day 90. Global disability within a treatment group will be based on the percentage of responders, where a responder is a subject who experiences a favorable functional outcome determined by a sliding dichotomy for mRS scores, as shown in Table 7-1.

Table 7-1: Sliding Dichotomy Definition of Responder

Age	Prehospital LAMS 2-3	Prehospital LAMS 4-5
Age 79 or under	mRS 0-1	mRS 0-2
Age 80 or over	mRS 0-2	mRS 0-2

The mRS is a valid and reliable measure of global disability that has been widely applied for evaluating functional disability following a stroke. It is a scale used to measure functional disability (the degree of disability or dependence in daily activities) in people who have suffered a stroke. Scores for the mRS range from 0 to 6, with 0 indicating no residual symptoms; 5 indicating bedbound, requiring constant care; and 6 indicating death. The mRS will be obtained at acute stroke hospital discharge, Day 4 ± 1 day, Day 30 ± 7 days, and Day 90 ± 30 days. Premorbid mRS status will also be obtained retrospectively. If the Day 90 mRS score is missing, it will be imputed as described in Section 5.1.1. Deceased subjects will be included in the Day 90 analysis with an mRS score of 6.

The primary efficacy analysis will be conducted on the analysis population as determined by the interaction effect and proportionality test as described in Section 7.1.3.1. Subjects will be grouped by randomized treatment, regardless of treatment actually received.

Given the critical importance of the key CACI population, the primary efficacy analysis will also be conducted on this population as a key supportive analysis in the trial.

7.1.2 Statistical Hypothesis

The primary hypothesis is that administration of nerinetide will result in a higher rate of responders.

The primary hypothesis is: $H_0: \pi_{nerinetide} = \pi_{placebo}$ VS $H_a: \pi_{nerinetide} \neq \pi_{placebo}$

Where $\pi_{nerinetide}$ and $\pi_{placebo}$ are the nerinetide and placebo population proportions of responders at 90 days, defined as per Table 7-1.

7.1.3 Primary Efficacy Analysis

The primary efficacy outcome is the overall proportion of subjects experiencing a favorable functional outcome 90-days post-randomization; subjects who are responders as defined in Table 7-1 are said to have favorable functional outcomes.

The primary efficacy hypothesis will be tested using a generalized linear model (GLM), adjusting for EMS hub, age, and baseline LAMS score with a log link to directly estimate the relative risk (RR). This use of a log-binomial regression is consistent with 2010 recommendations to avoid overestimation of treatment effects via odds ratios.[12,13] This is to provide the best treatment effect estimate of the absolute difference in the primary outcome variable, as responses are expected to be relatively common and a direct odds ratio may overestimate the RR, and hence the absolute treatment effect, in such a model. If the binomial model fails to converge using a log link, the plan will be to revert to traditional logistic regression using a logit link function. Only main effects will be evaluated.

7.1.3.1 Primary Efficacy Analysis Population

As summarized in Section 1.1.2, a potentially important modifier of the effect of nerinetide is that of reperfusion of the ischemic territory. To align the FRONTIER analysis for this possibility, the following steps will be implemented in order to define the primary efficacy analysis population:

Step 1

Assessment of Interaction Between Treatment and use of Reperfusion Therapy in the mITT population. This will occur using a model that includes the covariates described for the primary efficacy analysis above (EMS hub, age, baseline LAMS score) as well as Reperfusion Therapy (yes/no) and the two-way interaction term for treatment and use of Reperfusion Therapy.

A) If the p-value of the interaction term, derived from a likelihood ratio test, between treatment with nerinetide and use of Reperfusion Therapy is > 0.3 for the drug group versus placebo, the primary efficacy analysis population will include the entire mITT population.

B) If the p-value of the interaction is ≤0.3, Step 2 below will be taken to report balance between the drug and placebo groups in the use of reperfusion therapies in the first 24 hours after hospital arrival.

Step 2

Report balance between the drug and placebo groups in the use of Reperfusion Therapies. This step is necessary because the use of reperfusion therapies is a post-randomization event. The proportion of enrolled subjects treated with a Reperfusion Therapy in the nerinetide group compared to the same proportion in the placebo control group will be evaluated using a Fisher's exact test.

A) If the p-value for the difference in proportion of use of Reperfusion Therapy among drug and placebo subjects is > 0.05, nerinetide and placebo groups will be deemed to be balanced with respect to this variable. In such instance, the primary efficacy analysis population will include only subjects who receive Reperfusion Therapy. A supportive analysis to the primary analysis will be conducted separately on the stratum of subjects who did not receive a Reperfusion therapy. Only main effects will be presented.

B) If the p-value for the difference in proportion of use of Reperfusion Therapy among drug and placebo subjects is ≤ 0.05, the nerinetide and placebo groups will be deemed to be unbalanced. In such an instance, the primary efficacy analysis population will be the entire mITT population. The primary efficacy analysis will be supported with a further analysis in which the main effect GLM adjusting for EMS hub, age, baseline LAMS score will also adjust for Reperfusion Therapy (yes/no). Only main effects will be presented.

The primary efficacy will be conducted on the primary efficacy analysis population at the 2-sided 0.05 significance level overall for the trial.

The log-binomial model can be implemented and relative risk estimated using SAS code similar to the following:

```
proc genmod data=adqs descending;
   where mittfl = 'Y';
   class hub trtpn(param=ref ref="[placebo value]");
   model mRS_resp = trtpn hub age lams / dist=bin link=log;
   estimate 'Trt vs placebo' trtpn -1 1 / exp;
run;
```

Should the log-binomial regression model fail to converge, the logistic regression model can be run using code similar to the following:

```
proc logistic data=adqs descending;
   where mittfl = 'Y';
   class hub trtpn(param=ref ref="[placebo value]");
   model mRS_resp = trtpn hub age lams / rl lackfit;
   oddsratio trtpn / cl=wald;
run;
```

Once the OR estimate is provided by the above logistic regression model, the following equation can be used to calculate the estimated adjusted RR:

$$RR = \frac{OR}{(1 - P_c) + (P_c \times OR)}$$

where P_c is the adjusted risk in the control (placebo) group.

The actual proportions for each treatment group will be reported, along with the estimated relative risk and associated p-value.

A supportive analysis to the primary analysis will be conducted with the primary analysis reapplied to the Per-Protocol population.

7.2 Supportive Analyses on the Primary Outcome

Additional analyses using the primary outcome variable will be performed as indicated for the sub-populations indicated in Table 2-1 provided that such additional analyses are deemed to be warranted. These analyses may be adjusted for the following covariates that are deemed to be clinically or prognostically important:

- Sex
- Type of diagnosis (hemorrhagic stroke / acute cerebral ischemia / Other)
- Race and ethnicity
- Side of stroke
- Whether the subject received reperfusion therapy was in the form of thrombolytics (e.g., tPA), endovascular thrombectomy, or none.
- Time since stroke symptom onset to the initiation of study drug administration

The same adjusted log-binomial regression as discussed for the primary outcome will be used for these supportive covariate-adjusted analyses in the primary efficacy analysis population. The model will include the variables included in the primary analysis and the above listed covariates of interest. Wald test p-values and corresponding 95% CIs for RR will be presented. These covariate analyses are supportive analyses.

7.3 Secondary Efficacy Analyses

All secondary analyses in this section will be conducted and presented using the approach outlined above for the primary efficacy analysis population and separately as a supportive analysis in the remaining populations.

7.3.1 Shift Analysis of Functional Dependence Across the Whole Distribution of mRS Scores

An important secondary analysis of the ordinal mRS scores will employ a proportional odds model (POM) to test the hypothesis that, among randomized subjects, those who are treated with nerinetide will show a shift in their mRS score distribution at 90 days or last rating, relative to the mRS distribution of the placebo subjects. The magnitude of the shift will be estimated as the common odds ratio (95% C.I.). Modified Rankin scores of 5 and 6 (bed-bound with severe disability, and death) will be collapsed into a single category representing severely limited functioning. If the Day 90 mRS score is missing it will be imputed as described in Section 5.1.1. A covariate-adjusted POM will be used to derive the common odds of improvement (i.e. the nerinetide vs. placebo “shift” in mRS score distributions). Model covariates will be the same as in the primary analysis.

SAS code similar to the following can be used to fit the POM to the subjects’ mRS score:

```
ODS graphics on;
```

```
proc logistic data=adqs;
   where mittfl = 'Y';
   class hub trtpn(param=ref ref="[placebo value]");
   model mRS = trtpn hub age lams;
   effectplot interaction(x=trtpn sliceby=mRS)/polybar;
   oddsratio trtpn;
run;
```

where "mRS" 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:

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

This means that regardless of how one chooses to dichotomize the mRS scale, the ratio of the odds of 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 [14,15].

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

In the event that the PO assumption, needed to proceed with the key secondary efficacy analysis described, determines that the assumption is invalid, this analysis will not be conducted and remaining secondary endpoints to be tested as listed in Section 7.3 will still be considered to be protected (overall trial alpha still controlled).

7.3.2 Mortality

Mortality rates, 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, will be analyzed by the logistic regression model as described for the primary efficacy analysis. Mortality rates will be assessed based on the safety population. If it is unclear whether a subject has died prior to Day 90, that subject will be imputed as described in Section 5.1.2. The odds ratio associated with the treatment effect, adjusted for the covariates as described for the primary endpoint analysis, will be presented with a 95% confidence interval and the associated Wald test.

7.3.3 Worsening of Stroke

The rates of worsening of stroke, defined as the proportion of subjects with a worsening of their strokes over the 90-day study period in the nerinetide and placebo control subjects, will be analyzed by the logistic regression method as described for the primary efficacy analysis.

Worsening of stroke is defined as progression, or hemorrhagic transformation, of the index stroke as documented in the study CRF (as an adverse event with the preferred term of either "Stroke in Evolution" or "Haemorrhagic transformation stroke") that (i) is deemed life-threatening and/or (ii) results in increased disability as gauged by a ≥4 point increase from lowest NIHSS during hospitalization and/or (iii) results in death. The odds ratio associated with the treatment effect, adjusted for the covariates as described for the primary endpoint analysis, will be presented with a 95% confidence interval and the associated Wald test.

7.3.4 National Institutes of Health Stroke Scale

The proportion of subjects with good neurological outcomes will be measured using NIHSS. The NIHSS is a standardized neurological examination method found to be a valid and reliable measure used to objectively quantify the impairment caused by a 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. The NIHSS will be administered at ED arrival (no more than four hours post-dose), 24 Hours, Day 4, and Day 90.

The NIHSS scores at Day 90 will be dichotomized into 0-1 (indicating a good neurological outcome) versus >1 (indicating otherwise). If the Day 90 NIHSS score is missing, it will be imputed as described in Section 5.1.3. Deceased subjects will be assigned a score of 42. The proportion of subjects achieving a good neurological outcome at Day 90 or the last rating in nerinetide versus placebo control subjects will be compared using the logistic regression model as described for the primary efficacy analysis. The odds ratio associated with the treatment effect will be presented with a 95% confidence interval and the associated Wald test statistic and p-value.

7.3.5 Barthel Index

The proportion of subjects with functional independence in activities of daily living will be measured using the Barthel Index (BI). The BI is an index of functional independence that has been found to be a valid measure of activities of daily living when employed in stroke trials**.** 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 Days 4, 30 and 90. Note that the original Barthel Index was a scale from 0-20, but this study will use the modified version. The modified Barthel Index simply multiplies the original scale by 5.

The Day 90 BI scores will be dichotomized at 0-90 (indicating otherwise) versus 95-100 (indicating independent functioning with activities of daily living). If the Day 90 BI score is missing, it will be imputed as described in Section 5.1.4. Deceased subjects will be assigned a score of 0. The proportion of subjects with independent functioning with activities of daily living at Day 90 or last rating in nerinetide versus placebo control subjects will be compared using the logistic regression model as described for the primary efficacy analysis. The odds ratio associated with the treatment effect will be presented with a 95% confidence interval and the associated Wald test statistic and p-value.

7.4 Tertiary Efficacy Outcome Analyses

Summary statistics for each tertiary efficacy endpoint will be tabulated by treatment group. The tertiary analyses will be considered exploratory, and will be conducted in the analysis populations defined in Table 2-1, if deemed appropriate.

7.4.1 Proportion of Subjects with Day 90 mRS ≤ 1 and mRS ≤ 2

The Day 90 mRS score will be dichotomized at mRS≤1 (indicating freedom from disability) vs. mRS>1 (indicating otherwise). Also, the Day 90 mRS score will be dichotomized at mRS≤2 (indicating functional independence) vs. mRS>2 (indicating freedom from dependence). If the Day 90 mRS score is missing it will be imputed as described in Section 5.1.1. The proportion of subjects with freedom from dependence/disability based on these dichotomies on Day 90 in nerinetide versus placebo control subjects will be compared using the logistic regression model as described for the primary efficacy analysis. The odds ratio associated with the treatment effect will be presented with a 95% confidence interval and the associated Wald test statistic and p-value.

7.4.2 EQ-5D-5L

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

For the EQ-5D-5L, the difference between nerinetide and placebo control subjects in the distribution of the EQ-VAS score at Day 90 or last rating 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 covariates used in the primary efficacy analysis. ANCOVA results will be summarized in a table.

7.4.3 Return to Baseline Function Within 24 Hours

The proportion of subjects in the Confirmed Acute Cerebral Ischemia Subset whose symptoms fully return to baseline function within 24 hours of symptom onset in nerinetide versus placebo control subjects will be compared using the regression model as described for the primary efficacy analysis. Return to baseline includes 1) diagnosis of transient ischemic attack (TIA) or 2) diagnosis of stroke with an NIHSS of 0 at 24 hours.

7.4.4 Subjects Who Receive Reperfusion Therapy

In the event that the primary efficacy analysis population is the mITT population, the number of randomized subjects who receive Reperfusion Therapy will be summarized based on use and type of Reperfusion Therapy received (thrombolytics, endovascular thrombectomy or both). Treatment groups will be compared using the regression model as described for the primary efficacy analysis.

7.4.5 Subjects Who Receive Thrombolysis

The number of randomized subjects who receive thrombolysis will be summarized. Treatment groups will be compared using the regression model as described for the primary efficacy analysis.

7.4.6 Favorable Outcome at 30 days

The proportion of responders at 30 days post-randomization will be analyzed using the regression model as described for the primary endpoint. If the Day 30 value is missing, the last rating prior to Day 30 will be used. A responder will be determined based on the sliding dichotomy in Table 7-1.

7.4.7 Academic Medical Center Linear Disability Score

The ALDS is a measure of physical disability. Selected items in the ALDS item bank will be formed into five 15-item sets, and the appropriate 15-item set will be administered to each individual subject according to mRS score at the Day 90 visit and will be analyzed further if warranted.

7.5 Other Analyses

In addition to the above analyses, several exploratory analyses will be performed if warranted.

The regression analysis described for the primary efficacy outcome will be performed if it is deemed to be appropriate on the following additional subgroups of the mITT population based on the following criteria:

1. Subjects who receive EVT (yes/no)
2. Among subjects who receive thrombolytics or EVT, those who receive it more than three hours from stroke symptom onset (>3 hrs. vs ≤3 hrs.)

The following on Primary Efficacy Analysis population

3. Age > 80 years of age
4. Age > 75 years of age
5. Sex (male vs. female)

6. Randomization within 1 hour of stroke onset vs. > 1 hour of stroke onset.
7. Severe stroke defined as baseline (ED arrival) NIHSS > 20 vs. NIHSS <= 20.
8. Severe stroke defined as LAMS 4-5 vs. < 4.
9. Outcomes by recognized ethnic and racial groups.

Additional sub-groups may be examined, but those specified above are of prior clinical interest. If the number of subjects in a certain subgroup is too small (e.g., < 16 subjects), the analysis in that subgroup may not be performed.

Effect sizes will be estimated as subgroup-specific odds ratios (±95% CIs) as follows. Separate regression models for the primary endpoint, with treatment group and the other covariates from the primary analysis, 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 forest plots.

Table 7-2:Summary of Inferential Efficacy Analyses

Endpoint Type	Endpoint	Primary Analysis*	Sensitivity Analysis*	Supportive Analysis*
Primary	Day-90 mRS (Responder vs. Non-responder)	Adjusted[1] log-binomial regression model, mITT[2], Single Imputation (SI)	If missingness > 5%, adjusted[1] log-binomial regression model, mITT[2], and 4 sensitivity analyses: • Multiple imputation • Worst score imputation • Best Score imputation • Tipping Point Analysis	Key supportive analysis: primary analysis on subjects with confirmed acute cerebral ischemia and subjects treated with thrombolytics. Others: Adjusted[1] log-binomial regression model, PP[2;] subjects without reperfusion therapy[3]; Confirmed ICH[3]. Log-binomial regression model in mITT[2] adjusted for: • Sex, • Type of diagnosis (hemorrhagic stroke / acute cerebral ischemia / Other), • Race and ethnicity, • Side of Stroke, • Type of reperfusion therapy received (thrombolysis / EVT/both), • Time since stroke symptom onset to the initiation of study drug
Secondary	Day-90 mRS shift (Ordinal)	Proportional odds model (POM), mITT[2], SI	If missingness > 5%, POM, mITT[2] and 4 sensitivity analyses: • Multiple imputation • Worst score imputation • Best score imputation • Tipping Point Analysis	POM, subjects with confirmed acute cerebral ischemia; subjects treated with thrombolytics; subjects without reperfusion therapy[3]; Confirmed ICH[3]
	Day 90 Mortality	Adjusted[1] log-binomial regression model, mITT[2] Missing data imputed based on Day 30 status	N/A	Adjusted[1] log-binomial regression model, subjects with confirmed acute cerebral ischemia; subjects treated with thrombolytics; subjects without reperfusion therapy[3]; Confirmed ICH[3]
	Worsening of Stroke	Adjusted[1] log-binomial regression model, mITT[2]	N/A	Adjusted[1] log-binomial regression model, subjects with confirmed acute cerebral ischemia; subjects treated with thrombolytics; subjects without reperfusion therapy[3]; Confirmed ICH[3].
	Day 90 NIHSS (≤1 vs. >1)	Adjusted[1] log-binomial regression model, mITT[2] Missing data imputed based	N/A	Adjusted[1] log-binomial regression model, subjects with confirmed acute cerebral ischemia; subjects treated with thrombolytics; subjects without

Endpoint Type	Endpoint	Primary Analysis*	Sensitivity Analysis*	Supportive Analysis*
		on the median score obtained at Day 90		reperfusion therapy[3]; Confirmed ICH[3]
	Day 90 Barthel Index (≤95 vs. >95)	Adjusted[1] log-binomial regression model, mITT[2] Missing data imputed based last observation carried forwards if score was obtained at Day 30 visit or later otherwise based on the median score obtained at Day 90	N/A	Adjusted[1] log-binomial regression model, subjects with confirmed acute cerebral ischemia; subjects treated with thrombolytics; subjects without reperfusion therapy[3]; Confirmed ICH[3]
Tertiary	Day 90 mRS 0-2	Adjusted[1] log-binomial regression model, mITT[2]	N/A	N/A
	Day 90 mRS 0-1[3]	Adjusted[1] log-binomial regression model, mITT[2]	N/A	N/A
	EQ-5D-5L[3]	Adjusted[1] log-binomial regression model, mITT[2]	N/A	N/A
	Return to baseline within 24 hours[3]	Adjusted[1] log-binomial regression model, mITT[2]	N/A	N/A
	% Received Reperfusion Therapy[3]	Adjusted[1] log-binomial regression model, mITT[2]	N/A	N/A
	% Received Thrombolysis[3]	Adjusted[1] log-binomial regression model, mITT[2]	N/A	N/A
	mRS Responder at Day 30[3]	Adjusted[1] log-binomial regression model, mITT[2]	N/A	N/A

*For analyses using the adjusted log-binomial regression model, traditional logistic regression using a logit link function will be used if the binomial model fails to converge
[1] Adjustment covariates include EMS hub, age, baseline LAMS score as well as Reperfusion Therapy (yes/no)
[2]The primary analysis population for efficacy will be as described in Section 7.1.3.1. In this table, "mITT" will be exchanged for the primary analysis population if it differs from mITT.
[3]Analysis to be conducted only if efficacy outcomes warrant further exploration

8.0 SAFETY ANALYSES

The assessment of safety will be conducted in the Safety Population. The main analyses will be frequency of SAEs and 90-day mortality.

8.1 Adverse Events

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

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

AEs of Special Interest (AESIs) will be defined as AEs related to hypotension, angioedema, or anaphylactoid reactions that occur within the first two hours following the end of study drug administration.

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

When reporting AEs 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 AEs by relationship to study treatment, if a given subject had more than one AE mapped to the same preferred term, then that AE will be counted once according to the highest level of relatedness (Related, Probably, Possibly, Unlikely/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 by preferred term
- Serious TEAEs (with start date 0-90) days by MedDRA SOC and by preferred term
- All TEAEs resulting in death by MedDRA SOC and by preferred term
- 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 (Probably, Possibly, Unrelated) by MedDRA SOC and by preferred term. Missing relationships will be assumed as 'related'. Unlikely assessments will be assumed as 'Unrelated'.
- All TEAEs resulting in discontinuation of treatment, by MedDRA SOC and by preferred term
- Serious TEAEs by relationship to study treatment (Probably, Possibly, Unrelated) by MedDRA SOC and by preferred term. Missing relationships will be assumed as 'related'. Unlikely assessments will be assumed as 'Unrelated'.
- TEAESIs with a start time occurring within 2 hours after end of drug infusion by MedDRA SOC and preferred term. AESIs are defined in Appendix 1: Listing of Treatment-Emergent AEs of Special Interest (TEAESI).

The following listings of AE occurrences will be provided:

- All AEs by MedDRA SOC and by preferred term
- All SAEs by MedDRA SOC and by preferred term
- All AEs leading to death by MedDRA SOC and by preferred term
- All AEs related to study drug by MedDRA SOC and by preferred term. "Related" will include Probably related, Possibly related and missing relationship.
- All deaths by treatment group
- All AEs resulting in discontinuation of treatment by MedDRA SOC and by preferred term

Serious adverse events will also be summarized for the following subgroups of the safety population if deemed necessary by the results of the primary efficacy analysis:

- subjects with confirmed ischemic stroke
- subjects with AIS with reperfusion therapy
- subjects with confirmed hemorrhagic stroke

Additional subgroup summaries may be generated as needed should any noticeable imbalances be observed within selected subgroups of interest.

8.2 Vital Signs

A summary (table) of blood pressure (systolic and diastolic) will be reported at pre dose (Visit 1), post dose (Visit 1), ED Arrival, at 24 hours and at Day 4. Absolute values and changes from Pre-dose to Post-dose, to ED Arrival, to 24 hours and to Day 4 will be summarized descriptively.

A summary (table) of heart rate will be reported at pre dose (Visit 1), at ED Arrival, at 24 hours and at Day 4. Absolute values and changes from Pre-dose to ED Arrival, to 24 hours and to Day 4 will be summarized descriptively.

A summary (table) of temperature and Oxygen saturation (SaO2) reported at ED Arrival and at 24 hours. Absolute values and changes from ED Arrival to 24 hours will be summarized descriptively.

A listing of all vital signs will be provided.

8.3 Laboratory Results

A summary (table) of complete blood count (platelets, hemoglobin and WBC), electrolytes (sodium, potassium and chloride) and chemistry (serum creatinine and serum glucose), INR, and PTT at ED Arrival and 24 hours will be provided.

Absolute values and change from ED arrival to 24 hour values for laboratory results will be summarized descriptively. Inferential statistics (i.e., p-values or CI) will not be provided for these data.

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

8.4 12-Lead Electrocardiogram Results

A summary (table) of ECG Results (Normal, Abnormal NCS, Abnormal CS-reported as AE) will be reported at ED Arrival. Subjects who are missing an ECG result will not be included in the analysis.

A listing of all ECG findings will be provided. This includes: ECG Results, Ventricular rate, PR interval, QRS duration, QT, QTc, P-axis and QRS- axis and Rhythm.

8.5 Prior and Concomitant Medications

All prior and concomitant medications collected on the CRF up to Day 4 will be summarized by ATC Level 2 and Preferred Term within treatment group as well as listed in by-treatment by-subject listings.

8.6 Additional Information of Interest

The following additional information will be reported:

- Date and time of stroke symptom onset, defined as the time last seen normal, time of start of study drug administration, time of thrombolytics start (if applicable), time of EVT start (if applicable).

8.7 Independent Data Monitoring Committee

An Independent Data Monitoring Committee (IDMC) is performing periodic safety reviews of the clinical data. Reviews have already occurred after 25 and 50 subjects were enrolled and reached their 90-day study visit. A further review will occur after 300 subjects have reached their 90-day study visit. The Independent Statistical Group will generate safety reports, which will include cumulative summary statistics; subject status in the study (e.g., number completed Day 90 visits); baseline characteristics; safety data, including adverse events (AEs) and serious adverse events

(SAEs) by AE Preferred Term, severity, and relatedness to the study medication and discontinuations due to AEs. Two versions of these safety reports will be created – an open (blinded) report to be distributed to the Trial Executive Committee and the IDMC, and a closed (unblinded) report to be distributed only to the IDMC. The closed reports will be forwarded to the Trial Executive Committee following database lock and unblinding at the end of the study.

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

The unblinded Independent Statistical Group will be sequestered from the Project Team, steering committee and investigators. The Independent Statistical Group will produce the IDMC Safety Reports and provide them to the IDMC members. The reports to the IDMC will be provided prior to the meeting. A list of planned tables listings and figures to be included in the safety and efficacy reports are provided in a separate document "FRONTIER Master List of Tables, Listings and Figures".

The Statistical Group is responsible to:

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

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

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

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 • Generalized oedema • Hypersensitivity • Laryngeal obstruction • Localized 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 • Generalized 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 generalized • Rash • Rash erythematous • Rash generalized • Rash pruritic • Renal failure • Respiratory arrest • Respiratory distress • Respiratory failure • Sensation of foreign body • Tachypnoea • Cardiac arrest • Cardio-respiratory arrest • Cardiovascular insufficiency

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