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

PROTOCOL NA-1-007, Version 9.0
(AMENDMENT #8)
ISSUE DATE: 19th February 2019

Health Authority File Number: EudraCT number 2016-001826-33
clinicaltrials.gov number NCT02930018

1 GENERAL INFORMATION

1.1 Signatures of Approval

Protocol No: NA-1-007

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

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 Guideline for Good Clinical Practice (ICH-GCP), the Tri-Council Policy Statement (2), where applicable.

Digitally signed by Michael D Hill
Date: 2019.02.27 19:23:16 -07'00'

27 Feb 2019

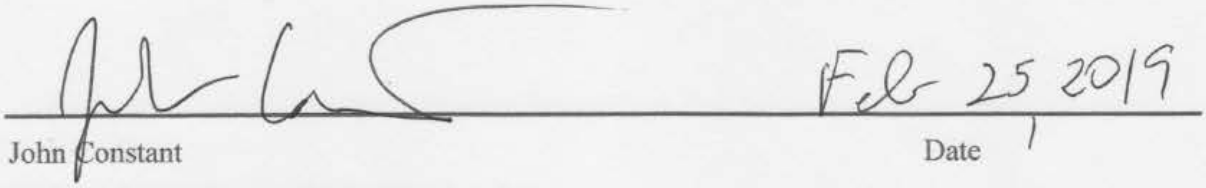
Michael D. Hill, M.D., MSc, F.R.C.P.C. — Date

Director, Calgary Stroke Unit

Feb 22nd 2019

Michael Tymianski, M.D., Ph.D., F.R.C.S.C. — Date

President and CEO, NoNO Inc.



Feb 25 2019

John Constant — Date

Statistical Consultant, PRA International

Table of Contents

Tables and Figures

1.2 List of Abbreviations

3PVO	Permanent Occlusion 3 Pial Vessels
ABC	Airway, Breathing, Circulation
ADC	Apparent Diffusion Coefficient
AIS	Acute Ischemic Stroke
ANCOVA	Analysis of Covariance
ANOVA	Analysis of Variance
aPTT	Activated Prothrombin Time
AE	Adverse Event
ASA	Acetylsalicylic Acid
ASPECTS	Alberta Stroke Program Early Computerized Tomography Score
β-hCG	Beta-human Chorionic Gonadotropin
BI	Barthel Index
BNT	Boston Naming Test
BP	Blood Pressure
Ca	Calcium
CBC	Complete Blood Count
CBV	Cerebral Blood Volume
CFR	Code of Federal Regulations
CI	Confidence Interval
CRF	Case Report Form
CRU	Clinical Research Unit
CT	Computed Tomography
CTA	Computerized Tomographic Angiography (multiphase or dynamic)
CTP	Computerized Tomographic Perfusion
DWI	Diffusion Weighted Imaging'
DVT	Deep Vein Thrombosis
ECG	Electrocardiogram
e-CRF	Electronic Case Report Form
ED	Emergency Department
EDC	Electronic Data Capture
EMA	European Medicines Agency
EVT	Endovascular Treatment
FDA	Food and Drug Administration
FLAIR	Fluid Attenuated Inversion Recovery
HIA	Health Information Act
HIPAA	Health Insurance Portability and Accountability Act
HIV	Human Immunodeficiency Virus
IA	Intra-arterial
ICA	Internal Carotid Artery
ICH	Intracranial Hemorrhage
ICH-GCP	International Conference on Harmonization-Good Clinical Practice
IDMC	Independent Data Monitoring Committee
INR	International Normalized Ratio
IOML	Inferior Orbitomeatal Line

IRB	Institutional Review Board
ITT	Intent-to-treat
IVH	Intraventricular Hemorrhage
IV	Intravenous
LAR	Legally Authorized Representative
MAP	Mean Arterial Pressure
MCA	Middle Cerebral Artery
MCAO	Middle Cerebral Artery Occlusion
MedDRA	Medical Dictionary for Regulatory Activities
MI	Myocardial Infarction
MIPS	Maximum Intensity Projections
MoCA	Montreal Cognitive Assessment
MR	Magnetic Resonance
MRI	Magnetic Resonance Imaging
mRS	Modified Rankin Scale
MRP	Magnetic Resonance Perfusion
Na+	Sodium
NaCl	Sodium Chloride
NCCT	Non-contrast Computed Tomography Scan
NHPSS	Non-human Primate Stroke Scale
NIHSS	National Institutes of Health Stroke Scale
NINDS	National Institute of Neurological Disorders and Stroke
NMDA	N-methyl D-aspartate
NMDAR	N-methyl D-aspartate Receptor
nNOS	Neuronal Nitric Oxide Synthase
NO	Nitric Oxide
O-F	O'Brien-Fleming
OLS	Ordinary Least Squares
OR	Odds Ratio
PEG	Percutaneous Endoscopic Gastrostomy
pH	Potential Hydrogen
PI	Principal Investigator
PIPEDA	Personal Information and Portable Electronic Documents Act
PK	Pharmacokinetic
pMCAO	Permanent Middle Cerebral Artery Occlusion
PP	Per Protocol
PSD-95	Post-synaptic Density 95 Protein
REB	Research Ethics Board
RNA	Ribonucleic Acid
RR	Risk Ratio
SAE	Serious Adverse Event
SAH	Subarachnoid Hemorrhage
SaO_2	Oxygen Saturation
SAP	Statistical Analysis Plan
SBP	Systolic Blood Pressure

SDH	Subdural Hematoma
SNAP	Sunnybrook Hemispatial Neglect Procedure
SOC	System Organ Class
SOP	Standard Operating Procedures
SUSAR	Suspected Unexpected Serious Adverse Reaction
TIA	Transient Ischemic Attack
TICI	Thrombolysis in Cerebral Infarction Score
TIMI	Thrombolysis in Myocardial Infarction
tMCAO	Transient Middle Cerebral Artery Occlusion
tPA	Tissue Plasminogen Activator (generic name = alteplase)
USA/US	The United States of America
WFNS	World Federation of Neurological Surgeons
WHO	World Health Organization

2 Study Synopsis

Trial Objectives	The primary objective is to determine the efficacy of the neuroprotectant, NA-1, in reducing global disability in subjects with major acute ischemic stroke (AIS) with a small established infarct core and with good collateral circulation selected for rapid endovascular revascularization. The secondary objectives are to determine the efficacy of NA-1 in: • Reducing functional dependence • Improving neurological outcome • Improving activities of daily living • Reducing mortality rate The leading safety objectives are to determine the effect of administering a dose of 2.6 mg/kg (up to a maximum dose of 270 mg) intravenous (IV) infusion of NA-1 to subject with acute stroke who are selected for endovascular revascularization on serious adverse events (SAEs) and 90-day mortality.
Trial Design	This study is a Phase 3, randomized, multicentre, blinded, placebo-controlled, parallel group, single-dose design. Subjects harboring an acute ischemic stroke and who are selected for endovascular revascularization in accordance with local institutional practices and who harbor a small established infarct core and with good collateral circulation will be given a single, 2.6 mg/kg (up to a maximum dose of 270 mg) intravenous dose of NA-1 or placebo as soon as they are deemed to have met the enrollment criteria and with the intention of starting administration within 30 minutes of randomization. The randomization will be by stochastic minimization to balance baseline factors.
Subjects	Up to 1120 male and female subject will be enrolled. <u>Inclusion Criteria</u> 1) Acute ischemic stroke (AIS) for immediate endovascular treatment 2) Age 18 or greater. 3) Onset (last-seen-well) time to randomization time within 12 hours. 4) Disabling stroke defined as a baseline National Institutes of Health Stroke Score (NIHSS) > 5 at the time of randomization. 5) Pre-stroke (24 hours prior to stroke onset) independent functional status in activities of daily living with modified Barthel Index (BI) > 90 (95 or 100). Patient must be living in their own home, apartment or seniors lodge where no nursing care is required. 6) Confirmed symptomatic intracranial occlusion, based on multiphase or dynamic computerized tomographic angiography (CTA), at one or more of the following locations: Intracranial carotid T/L, M1 middle cerebral artery (MCA). Functionally, when defining the M1 or the M2, the bulk of the MCA territory must be ischemic.

	7) Non-contrast computed tomography (NCCT) and CTA* for trial eligibility performed or repeated at ESCAPE-NA1 stroke centre with endovascular suite on-site. 8) Endovascular treatment with declared first endovascular approach as either stent retriever or aspiration device, and intended to be initiated (arterial access) within 60 minutes of baseline/qualifying NCCT and to first recanalization of 90 minutes. Study drug intended to be administered within 60 minutes of the baseline/qualifying NCCT. 9) Signed informed consent from subject or legally authorized representative or, if required to enable inclusion by applicable national laws and regulations and the applicable independent review boards/Ethics Committee requirements for obtaining consent, from the investigator after consultation with an independent physician who is not otherwise participating in the trial. *As per the List of Abbreviations (Section 1.2), all references to CTA indicate a multiphase or dynamic CTA. <u>Exclusion Criteria</u> 1) Evidence of a large core of established infarction defined as ASPECTS 0-4. 2) Evidence of absence of collateral circulation on CTA (Collateral score of 0 or 1). 3) Intent to use any endovascular device other than a stent retriever or clot aspiration device or intra-arterial medications as the initial thrombectomy approach. 4) Intent to use any intravenous thrombolytic other than alteplase if intravenous thrombolysis is planned. 5) No femoral pulses, very difficult endovascular access or extreme tortuosity of great vessels that is predicted to result in an inability to deliver timely endovascular therapy. Direct common carotid or radial/brachial/axillary access is permissible. 6) Estimated or known weight > 120 kg or < 45 kg. 7) Pregnancy; if a woman is of childbearing potential a urine or serum beta human chorionic gonadotropin (β-hCG) test is positive, or breastfeeding. 8) Severe contrast allergy or absolute contraindication to iodinated contrast preventing endovascular intervention, including any contraindications listed in the prescribing information approved by local authorities (e.g., patients with decompensated heart failure as a contraindication for the use of VISIPAQUE™ 270 in Germany). 9) Clinical history, past imaging or clinical judgment suggests that the intracranial occlusion is chronic or there is suspected

	intracranial dissection such that there is a predicted lack of success with endovascular intervention. 10) Prior enrolment in the ESCAPE-NA1 trial or prior receipt of NA-1 for any reason. 11) Severe known renal impairment defined as requiring dialysis (hemo- or peritoneal) or if known a creatinine clearance < 29 mL/min. 12) Patient has a severe or fatal comorbid illness that will prevent improvement or follow-up. 13) Patient cannot complete follow-up treatment due to co-morbid non-fatal illness or they are known to be a visitor to the city or any other known reason for which follow-up would be impossible (e.g. incarcerated in a federal prison). 14) Participation in another clinical trial investigating a drug, medical device, or a medical procedure in the 30 days preceding study inclusion.
Countries	Canada, Ireland, Sweden, U.S., South Korea, United Kingdom, Australia, Germany
Treatment	NA-1 2.6 mg/kg (up to a maximum dose of 270 mg, or matching normal saline placebo volume) will be administered as a single 10 ± 1 minute intravenous infusion in the upper or lower extremity using an infusion pump starting after randomization. All subjects will undergo attempted endovascular recanalization therapy with the intended endovascular approach being either using a stent retriever or clot aspiration device and receive best medical care according to modern acute stroke care guidelines. Stent retrievers or aspiration devices will be used according to current local jurisdictional guidelines.
Consent	Signed informed consent will be obtained prior to any protocol-specific procedures from the subject or legally authorized representative or, if required to enable inclusion by applicable national laws and regulations and the applicable independent review boards/Ethics Committee requirements for obtaining consent, from the investigator, after consultation with an independent physician who is not otherwise participating in the trial. If the original process involved anyone other than the subject and if required by local standards, consent will be sought for the remaining procedures from the subject once they are deemed to have regained capacity.
Randomization Method	Treatment will be assigned using 1:1 randomization (placebo:NA-1) with a stratification and minimization algorithm to minimize the

	contribution of imbalances in baseline factors and non-randomized therapies (e.g., alteplase use) to differences between treatment groups.
Duration of Treatment	This study consists of one 90-day study period for each subject. Subjects will be hospitalized for care after their acute stroke according to the current standard of care. Subjects are required to return to clinic on Days 30 and 90 for end-of-study procedures.
Laboratory Tests	In order to support the assessment of patient safety baseline (pre-dose) and post dose hematology and chemistry laboratory tests will be collected. At baseline, and at 24 ± 12 hours after study drug infusion and termination of EVT blood work will be evaluated which include: complete blood count (CBC), electrolytes, international normalized ratio (INR), activated prothrombin time (aPTT), serum creatinine and serum glucose. Other laboratory or point-of-care testing may be performed at the discretion of the attending physicians and team. If the subject is female and is of childbearing potential, a pregnancy test (urine or serum point-of-care pregnancy test) must be completed and a negative test result obtained prior to inclusion in the trial. Electrocardiograms will also be collected and reviewed at baseline (pre-dose, unless impeding access to timely intervention) and at 24 hours.
Assessment of Efficacy and Power	The primary efficacy outcome variable for the pivotal assessment of efficacy for regulatory submission purposes is the overall proportion of subjects experiencing a favorable functional outcome 90 days post-randomization, defined as a score of 0 to 2 on the modified Rankin Scale (mRS). These subjects are defined to be responders. Assuming a 52% overall responder rate for the placebo group, there will be an approximately 80% power to detect an 8.7% absolute effect difference between response rate (proportion of responders, with Day 90 mRS in the range 0 to 2) with NA-1 and placebo, at alpha level 0.05, 2-sided with a planned sample size of 1076 evaluable subjects, randomized 1:1, per group, accounting for a single interim analysis when 600 subjects have completed their 90 day follow up visit information (600 subjects with primary endpoint assessments) with O'Brien-Fleming alpha-spending function stopping boundary for overwhelming efficacy and a non-binding 1% conditional power futility stopping boundary (EaST® V6.3). The sample size will be inflated approximately 4% to N=560 per group to account for loss-to-follow-up and drop-outs. The primary hypothesis to be tested is that administration of NA-1 will result in an increase in the proportion of subjects with independent functioning on the mRS (as defined by a score of 0-2) at Day 90. The

	primary analysis will be a Wald test for treatment group difference in the primary outcome from a logistic regression including treatment and the following important prognostic factors: intravenous alteplase treatment, intended initial endovascular approach as well as age, sex baseline NIHSS score, baseline ASPECTS score, occlusion location and site. The primary efficacy analysis and secondary endpoint analyses will be conducted on the intent-to-treat (ITT) population, defined as all subjects randomized into the trial with grouping by randomized treatment, regardless of treatment actually received. Deceased subjects will be included in the ITT population with a mRS score of 6. A key secondary outcome analysis is planned to evaluate a shift of one or more categories to reduced functional dependence analyzed across the whole distribution of scores on the mRS at Day 90 or the last rating. This secondary outcome will be an adjusted analysis using a proportional odds model to derive the common odds of improvement ("shift") along the mRS scale. Adjustment will include all of the variables used in stratification (intravenous alteplase treatment, intended initial endovascular approach) and in the minimization algorithm (age, sex, baseline NIHSS score, baseline ASPECTS score, occlusion location, and site). The proportional odds assumption will be tested. Other secondary outcomes include: 1) Proportion of subjects with good neurological outcome, as defined by a score of 0-2 on the NIHSS at Day 90 or the last rating. 2) Proportion of subjects with functional independence in activities of daily living, as defined by a score of ≥ 95 on the BI at Day 90 or the last rating. 3) A reduction in mortality rate, as defined by event rate (%) for mortality over the 90 day study period 4) Proportion of subjects with functional independence, as defined by a score of 0-1 on the mRS at Day 90 or the last rating.
Assessment of Safety	For the safety analysis, the frequency of SAEs, 90-day mortality, adverse events (AEs), discontinuations due to AEs. As well, baseline and post-dose study drug vital signs, laboratory (hematology and chemistry) and electrocardiogram (ECG) findings will be analyzed.
Independent Data Monitoring Committee	An Independent Data Monitoring Committee (IDMC) will perform periodic safety reviews of the clinical data. The reviews will occur once 100, 300 and 600 subjects have reached their Day 90 final study visit. An efficacy interim analysis (after approximately 600 subjects complete

	the Day 90 follow-up) will be conducted using the alpha spending function method[1] with O'Brien and Fleming[2] type stopping boundary for efficacy and a non-binding conditional power boundary for futility. The trial may be stopped for overwhelming efficacy or futility at the interim analysis if the test statistic crosses the O'Brien-Fleming (O-F) or conditional power boundary.
Bioanalytical Methods	The plasma concentrations (immunogenicity) of NA-1 from a subset of up to 250 subjects from sites located in Canada and the USA will be analyzed using a validated direct ELISA assay method. Pharmacokinetic assessments from a subject of up to 100 subjects from a subset of sites located in Canada and the USA will be evaluated.

Table 2-1: Schedule of Assessments

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

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

★★Vitals signs (BP, HR only) should be recorded immediately before and after completion of the study drug infusion, temperature will be taken at baseline per standard of care.

¶ Historical (pre-stroke) mRS score can be collected at any time and will be reported on the 24h CRF.

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

* At baseline estimated or actual weight

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

‡ Blood should be drawn at baseline, but results are not required prior to randomization.

§For ECG, if treating physician deems that pre-treatment ECG impedes access to timely care, ECG must be completed within 6 hours of randomization.

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

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

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

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

d = days; h = hours

3 Coordinating Centre, Sponsor, Investigators and Facilities

Coordinating Centre	University of Calgary Foothills Medical Center 1403 29th Street NW Calgary, AB T2N 2T9
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Project Director:	redacted NoNO Inc
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Co-Coordinating Investigators	Mayank Goyal, M.D., F.R.C.P.C. Director of Research, Department of Diagnostic Imaging Bijoy K. Menon, MD, DM, MSc Assistant Professor Departments of Clinical Neurosciences, Radiology and Community Health Sciences

Data Management Center:	Clinical Research Unit (CRU) University of Calgary
Imaging Management Centre:	Calgary Image Processing and Analysis Center (CIPAC), University of Calgary

4 BACKGROUND INFORMATION

4.1 Rationale

The rationale for this study is the following:

1) There is no convincing evidence from randomized controlled trials that neuroprotection can be of clinical benefit to patients with AIS. There is however, extensive preclinical evidence that neuroprotection is of greatest benefit for improving functional outcome in studies employing experimental animal stroke models in which stroke is followed by reperfusion, as compared with stroke models in which arterial vessel occlusion is undertaken without reperfusion.
2) The ESCAPE and other recent trials of endovascular thrombectomy for AIS have demonstrated that modern acute endovascular therapy can rapidly recanalize blocked major arteries in patients with acute stroke arising from large artery occlusion with a low complication rate and high complete reperfusion rates.[3-7] This paradigm replicates the preclinical animal ischemia-reperfusion models in which neuroprotectants have the highest demonstrated efficacy.
3) The neuroprotectant, NA-1, has been demonstrated to be highly effective in reducing stroke size and improving the functional outcome of experimental animals subjected to acute stroke, including rats and primates. NA-1 is more effective in reducing infarct size and improving functional outcome in models of ischemia-reperfusion, as compared with permanent arterial occlusion[8,9,10].
4) NA-1 has an excellent safety profile in preclinical animal studies, a human Phase 1 trial, and a human Phase 2 study (the ENACT trial).[11] Patients in the ENACT trial were also individuals who were being subjected to an endovascular procedure (intracranial aneurysm repair) and to treatment with NA-1 or placebo.
5) NA-1 is the only neuroprotectant to have been shown to reduce ischemic brain damage in humans having demographics similar to those of stroke patients.
6) The inclusion/exclusion criteria of the ESCAPE trial[3] were successful in identifying patients with salvageable brain (an ischemic penumbra). Modern endovascular techniques have extremely high rates of safe and effective reperfusion. This makes the ESCAPE patient selection process and use of endovascular recanalization an ideal clinical paradigm to replicate prior preclinical animal studies that support the effectiveness of the neuroprotectant, NA-1.
7) There is a compelling need to develop neuroprotectants in order to increase the proportion of patients who may benefit from recanalization therapies. These agents could improve the outcomes of those who receive endovascular recanalization, and make more patients into candidates for endovascular or pharmacological recanalization treatments.

4.2 The Neuroprotectant, NA-1

NA-1 is a synthetic, cell-permeant eicosapeptide (20 amino acids) that perturbs protein-protein interactions on the cytosolic surface of the cell membrane mediated by post-synaptic density 95 protein (PSD-95)[12], an abundant protein localized in post-synaptic densities of central nervous system neurons. It may provide significant benefit for the treatment of acute cerebral ischemia if administered to stroke patients who present to medical attention before infarction is complete. The rapid progression of irreversible brain injury in most acute strokes implies a short window of clinical efficacy of any treatment, including NA-1. The ability to identify patients with salvageable brain using the criteria used in the ESCAPE trial provides an opportunity to target patients who

may have the greatest benefit from neuroprotection, and to enhance further the impact of reperfusion therapies[3]. Our preclinical and clinical data support this notion.

NA-1 is composed of two parts: a 9-amino acid active substance that binds to PSD-95, and an 11-amino acid sequence derived from the human immunodeficiency virus (HIV)-1 Tat protein which permits NA-1 to penetrate through the blood-brain barrier and enter neuronal target cells. PSD-95 couples transmembrane proteins such as the N-methyl-D-aspartate (NMDA) subtype of glutamate receptors to various intracellular signaling enzymes that mediate the neurotoxic sequelae of NMDA receptor activity. Specifically, NA-1 inhibits the interaction between PSD-95 and the GluN2B subunit of the NMDA receptor (NMDAR), thus uncoupling NMDARs from downstream neurotoxic signaling enzymes and preventing or limiting the onset of excitotoxicity that is associated with AIS (Figure 1). NA-1 has no known effect on the electrophysiological aspects of NMDAR channel function, but results in decreases in pro-death signals downstream of NMDARs such as reduced production of the toxic free radical nitric oxide (NO). [13]

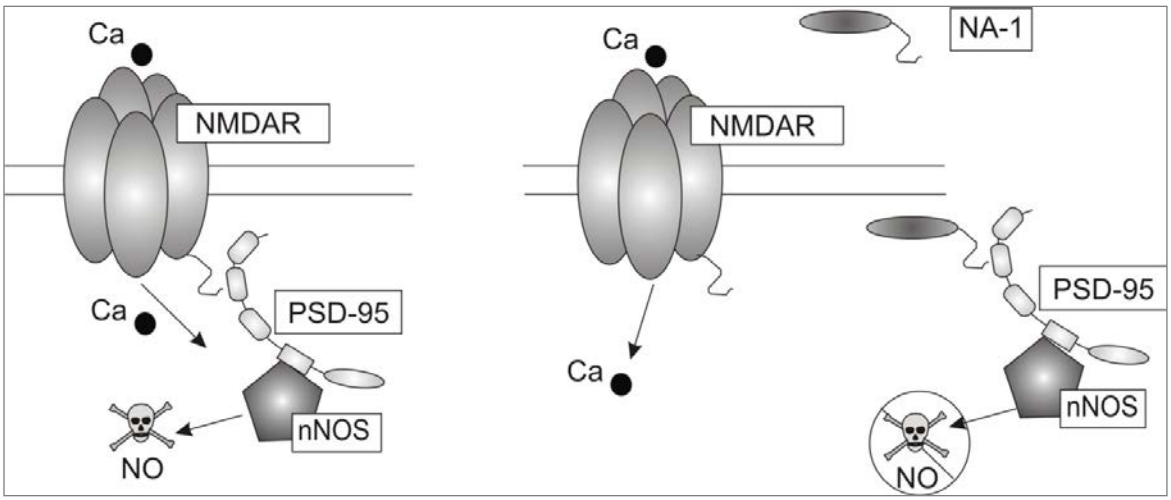


Figure 1: Inhibition of NO production by NA-1 via perturbation of NMDAR-PSD-95 interactions [Ca=calcium; nNOS=neuronal nitric oxide synthase]

4.3 Summary from Nonclinical Studies and Clinical Trials

NA-1 has been shown to be a promising neuroprotectant in rats and in non-human primates exposed to experimental strokes when it is administered within three hours of stroke onset[8,13]. It is also effective in reducing iatrogenic stroke burden in humans subjected to endovascular aneurysm repair as measured by a reduction in the number of new lesions visualized by magnetic resonance imaging (MRI)[11]. In the human study, NA-1 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 benefit to patients with AIS[14]. All these studies had in common the fact that NA-1 was administered in a paradigm in which stroke damage was not yet complete. See Figure 1**: Inhibition of NO production by NA-1 via perturbation of NMDAR-PSD-95 interactions** [Ca=calcium; nNOS=neuronal nitric oxide synthase]

4.3.1 Nonclinical Studies

4.3.1.1 NA-1 Effect on Infarct Size and Volume in Rodent Models of Stroke

Several *in vivo* pharmacology studies have been conducted with NA-1 in rodents to evaluate the effect of NA-1 on infarct size and volume in models of stroke involving either transient or permanent occlusion of intracranial arteries, and effects on sensory, emotional, and cognitive deficits. The results of the rodent studies demonstrated that NA-1 is delivered to the brain of an intact animal following systemic exposure via intravenous administration, and also appears to accumulate at sites of brain injury when given intravenously after animals had been subjected to a cortical stroke induced by permanent occlusion 3 pial vessels (3PVO model)[9]. When administered intravenously, post-insult treatment with NA-1 (0.08 to 7.60 mg/kg) in a transient stroke model produced by middle cerebral artery occlusion (tMCAO) is effective in both permanently reducing stroke size, and in improving neurological function even when treatment is administered as late as three hours after stroke onset. There was no effect on normal central nervous system function. Rats treated with NA-1 had reduced motor, sensory, emotional, and cognitive deficits, as well as faster recovery of pre-surgery weight. NA-1 has been shown to be efficacious in models of permanent stroke in rats at a dose of 7.6 mg/kg when administered one-hour post-insult[9]. Furthermore, Fisher and colleagues found that administration of NA-1 in a rat model of permanent middle cerebral artery occlusion (pMCAO) "froze" the ischemic penumbra measured using MRI[15]. In other words, NA-1 administration in the face of an evolving ischemic injury halted the progression of further brain damage as of the time it was administered.

4.3.1.2 Effect of NA-1 in Reperfused MCAO Model of AIS in Non-Human Primates (30-Day Study)

To test whether NA-1 is neuroprotective in acute AIS in a gyrencephalic species, a blinded, randomized trial of NA-1 versus drug vehicle in a tMCAO model in 20 cynomolgus macaques was completed[8]. NA-1 2.6 mg/kg or drug vehicle were administered in blinded fashion 60 minutes following middle cerebral artery occlusion (MCAO). The MCA was reperfused 90 minutes following occlusion. Stroke volume on 24 hour MRI diffusion weighted imaging (DWI) was statistically significantly reduced by over 50% in NA-1-treated animals as compared with placebo animals and this effect was maintained on the Day 30 MRI. There was also a significant improvement in Non-Human Primate Stroke Score (NHPSS) and significant improvements in sensory and motor function.

There were no differences in any of the physiological parameters [including mean arterial pressure (MAP)] for NA-1 and placebo treated animals, with the exception of temperature at the time of MRI completion (36.5ºC ± 0.27 in placebo treated animals versus 37.4ºC ± 0.16 in NA-1 treated animals, p=0.015).

4.3.1.3 Effects of NA-1 on Infarct Volume and Gene Transcription Response in pMCAO Model of AIS in Cynomolgus Macaques

To test the neuroprotective effect of NA-1 on infarct volume and gene transcription response, six macaques underwent pMCAO, with NA-1 or placebo treatment administered five minutes after ischemia onset. DWI MRI scans were taken every 15 minutes. The volume of brain in which

DWI hyperintensity was detectable increased over time in both groups. However, treatment with NA-1 attenuated the rate of this increase by about twofold (p=0.019). Moreover, within the ischemic volume, DWI intensity in brains of NA-1-treated animals remained lower than that of untreated controls, suggesting that tissue within the infarct volume maintained better integrity[8].

Tissue was collected from the ischemic penumbras (adjacent to infarcted tissue) and from corresponding sites in the contralateral non-ischemic hemisphere at one and six hours after ischemia onset. Ribonucleic acid (RNA) was extracted and hybridized to whole-genome macaque arrays. Differential gene expression analysis revealed that treatment with NA-1 resulted in a lower proportion of down regulated genes and preservation of the capacity to up regulate genes at both one and six hours after stroke. Overall, the genome-wide survey suggested that neuroprotection with NA-1 preserved cellular functionality as gauged by the capacity for gene transcription in ischemic brain tissue[8].

4.3.1.4 Effect of NA-1 in Prolonged tMCAO Model of AIS (7-Day Study)

The hypothesis that NA-1, administered one hour into a 4.5 hour tMCAO in cynomolgus macaques (which is the limit of effectiveness of alteplase in a human reperfusion study[16]) would improve stroke neurological outcomes and reduce stroke volumes seven days following stroke was tested. Six animals received NA-1 (2.6 mg/kg IV) and six received placebo one hour following MCAO. The MCA was reperfused 4.5 hours after MCAO. There was a significant reduction in stroke volume (relative reduction of 25%) in the NA-1 group in comparison with the placebo group. Scores on the NHPSS were significantly improved in NA-1 treated animals at seven days[8]. There were no differences in any of the physiological parameters (including MAP) at any of the measured time points for the NA-1 versus placebo treated animals.

4.3.1.5 Effect of NA-1 Administered Three Hours After Onset of 3.5 Hour tMCAO in Cynomolgus Macaques (14-Day Study)

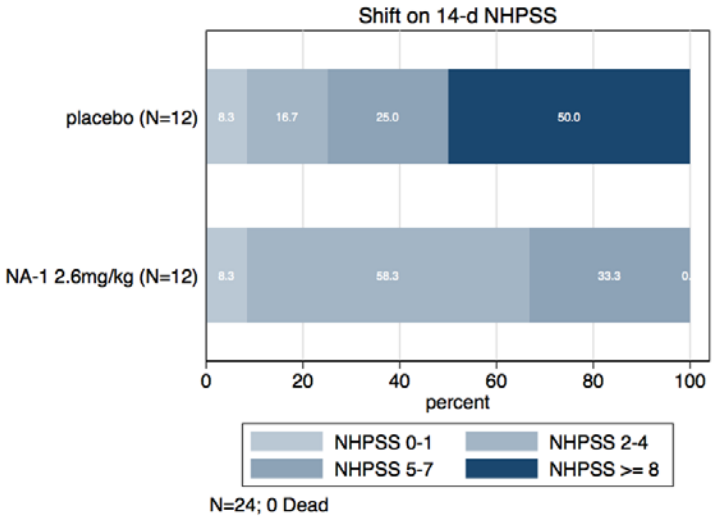
To test whether NA-1 is beneficial when administered later in the setting of a prolonged tMCAO, 24 cynomolgus macaques received a 10-minute infusion of NA-1 or placebo three hours after the onset of a 3.5 hour tMCAO. There were no mortalities. Final imaging and neurological assessments were conducted at 14 days. NA-1 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 NA-1 exhibited improved NHPSS scores throughout the 14-day observation period days [p=0.004, two-way repeated measures analysis of variance (ANOVA)] and trended to better performance in the six-well and the valley staircase tasks[8]. There were no statistically significant differences in any of the physiological parameters (including MAP) at any of the measured time points for the NA-1 versus placebo treated animals.

We have reviewed the outcomes using analyses analogous to those proposed in the current protocol, but using the available NHPSS scores instead of the mRS scores proposed in this current trial.

Analogous to our proposed analysis of the primary outcome using a dichotomy approach, the NHPSS was divided in a binary fashion defining good outcome as NHPSS 0-2 at 14 days. With this, we calculated the risk ratio (RR) = 3.0 (0.7-12.0), p = 0.193, with an absolute risk difference of: 0.33 (CI_{95} -0.02 – 0.69). Alternatively, using simple ordinary least squares (OLS) linear regression, NHPSS = -3.5*trt + 6.9, $p_{(\beta\text{-coeff})}$ = 0.005. So on average at 14 days, the NHPSS was 3.5 points less in the treated group.

By analogy to one of our proposed secondary analyses using a proportional odds model ('shift analysis'), we presented the outcome on the NHPSS divided in quartiles at 14 days. Note that the NHPSS shift here is calculated by quartiles of NHPSS and not by pre-specified groups.

Common odds ratio (OR; (proportional odds model) for shift along the NHPSS = 8.1 [Confidence Interval $(CI)_{95}$ 1.5-45.3)], p = 0.016.



4.3.1.6 Efficacy of NA-1 in Reducing the Number and Volumes of MRI-visible Strokes in a Cynomolgus Macaque Model of Micro-embolic Stroke

A study of the efficacy of NA-1 in reducing the number and volume of MRI-visible strokes in a cynomolgus macaque model of microembolic stroke was completed[10]. The purpose of the study was to evaluate NA-1 in an experimental setting that mimics the MRI-detectable strokes sustained by human patients as a result of endovascular brain aneurysm repair. The study evaluated the effects of an intravenous dose of NA-1 2.6 mg/kg on the volume and number of cerebral infarcts produced by an intra-arterial injection of 20 100µm polystyrene spheres in cynomolgus macaque monkeys, an experimental embolic stroke paradigm that produces a permanent occlusion of the affected brain vessels.

Ten cynomolgus macaques were randomized to receive NA-1 2.6 mg/kg or placebo. Following induction of anesthesia, each animal underwent insertion of a femoral angiography catheter and a cerebral angiogram was performed. Each animal then received an injection of 20 100µm polystyrene sphere emboli. At one hour following the embolic procedure, NA-1 (2.6 mg/kg) or placebo [equivalent volume of 0.9% sodium chloride (NaCl)] was infused intravenously in 5 mL of 0.9% NaCl over 10 minutes. Animals underwent diffusion and T2-weighted imaging at four hours after the injection of emboli. Twenty-four hours after the embolic procedure, the animals were re-anesthetized and returned to the MRI scanner for further DWI and T2 imaging. A further MRI at four weeks was utilized to confirm that DWI hyperintensities had resolved completely. After this 4-week washout period, each animal was crossed over to the opposite treatment group and the experiment was repeated.

All 10 animals completed the first iteration of the crossover trial. On the second round, three animals were lost due to an air embolus in a placebo animal, a post-operative aspiration in a placebo animal, and an anesthetic related cardiac arrest preceding surgery and dosing in the third. There were no significant differences in physiologic parameters between groups (including MAP). Animals treated with NA-1 exhibited markedly reduced stroke numbers and volumes as compared with placebo-treated animals. Numbers of total strokes were: 4.7 ± 0.16 and 12.75 ± 0.15 in NA-1 versus placebo respectively ($p<0.001$). Volumes of total strokes were: 28.4 ± 2.98 and 64.4 ± 3.41 in NA-1 versus placebo respectively ($p = 0.015$).

This study revealed that counting the number and/or volume of new, small lesions incurred by small intra-arterial emboli is a useful bio-assay that can detect the consequences of penetration of a study drug into target brain tissue and to measure its intended biological effect on reducing stroke damage.

4.3.1.7 Nonclinical Primary Pharmacology Summary

In summary, NA-1 is a novel peptide that has now been repeatedly shown to protect against ischemic damage following stroke in several rodent and non-human primate models. NA-1 targets intracellular protein interactions downstream of glutamate receptors, thus acting via a novel mechanism. Studies have demonstrated that, in addition, NA-1 does not affect the electrophysiological function of NMDAR channels. Therefore, unlike NMDA receptor antagonists, NA-1 is in a class of drugs that is not anticipated to have negative side effects that were ascribed to glutamate receptor antagonists.

4.3.2 Clinical Trials

4.3.2.1 Evaluating Neuroprotection in Aneurysm Coiling Therapy (ENACT) Trial

To test the feasibility of neuroprotection in the human brain based on the results in the non-human primate microembolic stroke study described above, a Phase 2 multicentre, randomized, fasting, double-blind, placebo-controlled, safety, tolerability, and efficacy study evaluating a single dose of intravenous NA-1 was recently completed in male and female patients undergoing endovascular repair of brain aneurysms (unruptured and ruptured)[11]. The primary objectives of the trial were to determine the safety and tolerability of a single intravenous dose of NA-1 (2.6 mg/kg) and the efficacy of NA-1 in reducing the volume of embolic strokes as measured by MRI imaging. The secondary objectives were to determine the efficacy of NA-1 in reducing the number of embolic strokes; procedurally-induced vascular cognitive impairment as measured by a battery of cognitive tests; and the frequency of large embolic strokes. The study consisted of one 30-day evaluation period.

A total of 185 subjects were enrolled in the study; 92 were dosed with NA-1 and 93 with placebo. In the NA-1 treatment group, 74 subjects (80%) had unruptured aneurysms on study entry and 18 (20%) were ruptured. The placebo group had 74 (80%) subjects with unruptured aneurysms and 19 (20%) with ruptured aneurysms. Six subjects (3.2%) did not complete the study: Two subjects died (both in the placebo group), one experienced an AE eventually leading to death (in the NA-1 group), two were lost to follow-up (in the NA-1 group), and one was discontinued due to protocol

non-compliance (failed to show up for the scheduled Day 30 visit; in the placebo group). Of 185 randomized subjects, 184 completed the post-procedure MRI scan (12-96 hours).

Overall, NA-1 reduced the mean number of lesions by three new lesions as evaluated by DWI (p=0.018) and by two new lesions as evaluated by fluid-attenuated inversion recovery (FLAIR; p=0.048) in comparison with placebo in All Randomized Subjects (subjects receiving any amount of study drug). The distribution of the volumes of new strokes was highly skewed, and did not follow a normal distribution. This was anticipated based on the non-human primate model of microembolic stroke study[10]. The median lesion volume was less than 1,000 mm^3. Two subjects with unruptured aneurysms exhibited large strokes (10,702 and 49,204 mm^3 by DWI volume) as a result of peri-procedural complications of endovascular repair, and both were in the NA-1 group. In both cases the major ischemic stroke occurred several hours after the procedure. The first patient was neurologically well for several hours post-procedure (placement of a Pipeline stent for a giant cavernous carotid aneurysm) and then developed aphasia. The second patient was neurologically well until the morning after his procedure (balloon-assisted coiling of an internal carotid artery aneurysm) when he developed right hemiparesis. Both had been treated with double antiplatelet therapy [acetylsalicylic acid (ASA) and clopidogrel]. Due to these two outliers, there was sufficient distortion of the mean volume of strokes in the NA-1 group such that the analysis of the impact of NA-1 on mean lesion volume was neutral in the analysis of all randomized subjects and only subjects with unruptured aneurysms. When subjects with strokes < 10 cc were considered (i.e., when the two outliers were excluded from analysis), a halving of lesion volume was observed in patients treated with NA-1. This effect was even more evident when the results were adjusted for the following pre-specified covariates: age, aneurysm status, use of adjunctive devices, groin puncture to infusion time, and the use of antiplatelet agents (p=0.009 for DWI and p=0.014 for FLAIR). Among the subgroup of subjects with ruptured aneurysms, NA-1 treatment reduced the volume of FLAIR and DWI lesions (FLAIR: p=0.023; DWI: p=0.015) and the number of FLAIR and DWI lesions (FLAIR: p=0.046; DWI p=0.027).

In terms of neurological outcomes at Day 30, there were no differences between the proportion of all randomized subjects with good outcomes (score of 0-1) on the NIHSS in the NA-1 and placebo groups at Day 30. Similarly, there were no differences between the proportion of subjects with independent functioning (score of 0-2) on the mRS in the NA-1 and placebo groups at Day 30. This was as expected because subjects that met the eligibility criteria were generally neurologically intact (unruptured aneurysms), or were of a good World Federation of Neurological Surgeons (WFNS) subarachnoid hemorrhage (SAH) grade (ruptured aneurysms). Baseline NIHSS and mRS scores were consistent with low levels of neurological impairment and disability (median NIHSS and mRS of 0), which leads to a floor effect in scale sensitivity. However, in the subjects with ruptured aneurysms (a subgroup with anticipated neurological impairments) treatment with NA-1 significantly increased the proportion of subjects with a good outcome on the NIHSS versus placebo at the 30-day follow-up (100.0% versus 68.4%, respectively; p=0.020). This was not accounted for by differences in post-treatment SAEs such as hydrocephalus, the need for cerebral spinal fluid diversion, or cardiopulmonary complications.

4.4 Summary of Known Potential Risks and Benefits

4.4.1 Phase 1 Study

A Phase 1, randomized, fasting, double-blind, placebo-controlled, safety, tolerability, and pharmacokinetic study evaluated single ascending doses of intravenous NA-1 in healthy male and post-menopausal or surgically sterile female human subjects (Biovail Contract Research Study 3301). The primary objectives of this study were to determine the safety and tolerability of single ascending intravenous doses of NA-1. Sixty-two normal, healthy, non-smoking male and post-menopausal or surgically sterile female subjects were dosed in eight cohorts. Subjects received a single dose of NA-1 or placebo on Day 1 of the study period, according to a randomization scheme. NA-1 was given as follows, with the dose increasing per cohort: 0.02, 0.08, 0.20, 0.375, 0.75, 1.50, 2.6 and 3.75 mg/kg of active drug, given as a 10 ± 1 minute intravenous infusion. Subjects were required to return on Days 7, 14 and 28 for interim and end-of-study procedures. There were no SAEs; the majority of noted AEs were mild in severity; no subjects failed to complete the study protocol; and no subjects withdrew from the study. Forty subjects experienced a total of 168 AEs. Thirty-four of the 46 active treated subjects (73.9%) experienced at least one AE, while six of 16 placebo treated subjects (37.5%) experienced at least one AE. Of the 168 AEs, 103 were listed as probably related to the study drug and five AEs were possibly related to the study drug. No severe AEs were reported while 160 of the 168 AEs were mild in severity and eight were moderate in severity. The most commonly reported AEs in subjects receiving any dose of NA-1 were flushing (22% of subjects), dry mouth (20%), feeling hot (20%), dizziness (17%), localized pruritis (15%), decreased blood pressure (BP;11%), generalized rash (9%), generalized erythema (9%), nausea (9%), increased blood glucose (9%), increased alanine aminotransferase (9%), oral hypoaesthesia (9%), paraesthesia (9%), generalized pruritis (7%), urine positive for white blood cells (7%), and localized rash (7%). The incidence of AEs in the 30 subjects receiving NA-1 at dose levels of 0.02, 0.08, 0.20, 0.375, 0.75 and 1.50 mg/kg was the same as that of the 16 placebo treatment subjects. Subjects in the 2.6 and 3.75 mg/kg dose groups experienced more AEs than subjects in the lower dose groups, though none was serious. Overall, NA-1 was well tolerated as a single intravenous dose in the range of 0.02 to 2.6 mg/kg, administered to healthy male and post-menopausal or surgically sterile female subjects.

4.4.2 Phase 2 Study (ENACT)

The Phase 2 ENACT study investigated the effect of a single intravenous dose of NA-1 2.6 mg/kg on reducing the volume and number of embolic strokes caused by the aneurysm repair procedure and in increasing the proportion of subjects with a good clinical outcome in 185 patients undergoing endovascular aneurysm coiling of unruptured or ruptured aneurysms[17]. In total, 724 treatment-emergent AEs were reported in 168 (91%) of all randomized subjects. A total of 388 AEs were reported in 85 of 93 subjects who received placebo (91%) and 336 AEs were reported in 83 of 92 subjects who received NA-1 (90%). The most frequently-reported individual AEs were: headache, reported in 40% of placebo subjects and 46% in NA-1 subjects; nausea (29% and 36%, respectively), vomiting (9% and 13%), procedural pain (11% and 2%; p=0.018), and hypotension (6% and 10%). Of the 388 events reported in placebo subjects, 208 (54%) were mild, 159 (41%) were moderate and 21 (5%) were severe. Of the 336 AEs reported in NA-1 subjects, 187 (56%) were mild, 121 (36%) were moderate and 28 (8%) were severe. Of the 388 AEs reported in placebo

subjects, 271 (70%) were unrelated, 96 (25%) were unlikely related, 21 (5%) were possibly related and 0 (0%) were probably related. Of the 336 AEs reported in NA-1 subjects, 251 (75%) were unrelated, 72 (21%) were unlikely related, 11 (3%) were possibly related and 2 (1%) were probably related. There were three deaths during this study, two in the placebo group and one in the NA-1 group. The SAEs leading to death were all severe and unrelated to study drug. Other than these three deaths, there were no other discontinuations due to AEs. There were 35 SAEs in 23 subjects: 24 SAEs in 14 placebo subjects and 11 SAEs in nine NA-1 subjects. None of the SAEs were assessed as related to study drug. Overall, there were very few abnormal clinically significant Hematology and Biochemistry results in both treatment groups. There were no noteworthy differences across time between treatment groups for any of the laboratory parameters. One placebo subject (1%) had an elevated histamine level postdose (16 nmol/L) versus five NA-1 subjects (5%) ranging from 10-15 nmol/L (normal range for males and females ≤ 10 nmol/L). In terms of vital signs, there were no noteworthy findings in mean heart rate across time between treatment groups. There were very few abnormal clinically significant 12-lead ECG results in the NA-1 and placebo groups. There were no cardiac AEs that were considered to be an SAE or significant AE in either treatment group. Cardiac AEs were infrequent in both treatment groups. Overall, NA-1 2.6 mg/kg was well tolerated and no safety concerns were identified in any of the patient groups.

4.4.3 Summary of Safety of NA-1

Overall, NA-1 administered as a single intravenous dose was well tolerated at doses up to and including 2.6 mg/kg, and no safety concerns have been identified in any of the patient groups in the clinical trials.

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

NA-1 2.6 mg/kg, up to a maximum of 270 mg (or matching placebo volume) is administered as a single approximately 10-minute intravenous infusion in the upper or lower extremity using an infusion pump initiated in the CT scan suite. The 2.6 mg/kg dose was chosen for this clinical trial because of (1) the safety profile observed in the previous Phase 1 and 2 clinical trials, (2) the observed capacity of this dose of NA-1 to reduce stroke tissue damage and to improve neurological function in rats and non-human primates exposed to experimental strokes when NA-1 was administered in animals exhibiting salvageable brain, and (3) the capacity of this dose to reduce stroke tissue damage and improve neurological damage in human subjects undergoing endovascular repair of brain aneurysms.

4.6 Target Patient Selection

Inclusion/exclusion criteria of all prior neuroprotection trials aimed at selecting subject who would respond to the neuroprotective study drug. Preclinical evidence indicates that neuroprotection is aimed at salvaging brain in the ischemic penumbra, defined as that part of the ischemic brain that can be prevented from progressing to infarction if an appropriate treatment is given. However, due to the failure of all prior neuroprotection trials, it is unclear whether the prior criteria used for selecting responders were appropriate.

The ESCAPE trial provides an unprecedented opportunity for testing neuroprotectants because its inclusion/exclusion criteria have successfully defined a patient population in whom brain salvage was achievable. This is therefore a de-facto patient population which is most likely to exhibit an ischemic penumbra, the target for neuroprotection, at the time of initiation of endovascular recanalization therapy. Additionally, the high rates of reperfusion achieved with endovascular recanalization recapitulate ischemia-reperfusion as closely as could be achievable in clinical settings. Extensive preclinical data indicate that neuroprotection is (a) most effective in ischemia reperfusion and (b) targeted to the penumbra. Thus it is most rational to evaluate neuroprotection in stroke patients who meet the ESCAPE criteria for endovascular recanalization therapy[3].

4.7 Endovascular Acute Stroke Therapy

Endovascular treatment for AIS has now been shown to be of clinical benefit in appropriately selected patients and is anticipated to become a standard of care. Six randomized trials – ESCAPE, EXTEND-IA, SWIFTPRIME, REVASCAT, MRCLEAN, THRACE - have demonstrated the benefit of endovascular therapy over best medical management.[3-7] The last of these, THRACE, has been presented only in abstract form. These publications cap a tremendous period of clinical activity after prior trials of endovascular stroke therapy had been neutral or negative.[18-20]

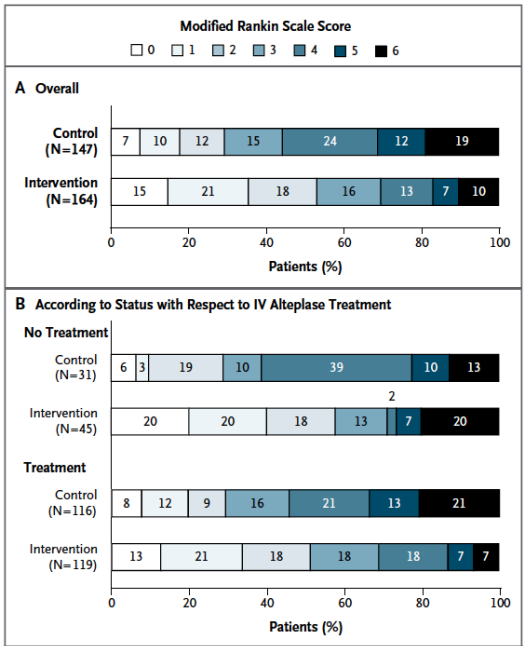


Figure 1. Scores on the Modified Rankin Scale at 90 Days in the Intention-to-Treat Population.

Scores on the modified Rankin scale range from 0 to 6, with 0 indicating no symptoms, 1 no clinically significant disability, 2 slight disability, 3 moderate disability, 4 moderately severe disability, 5 severe disability, and 6 death. Panel A shows the distribution of scores at 90 days in the intervention and control groups in the overall trial population. A significant difference between the intervention and control groups was noted in the overall distribution of scores (unadjusted common odds ratio, indicating the odds of improvement of 1 point on the modified Rankin scale, 2.6; 95% confidence interval, 1.7 to 3.8), favoring the intervention. Panel B shows the distribution of scores at 90 days in the intervention and control groups according to status with respect to intravenous (IV) alteplase treatment. In this analysis, there was no evidence of heterogeneity of effect (P=0.89 for interaction by the Wald test).

The trials were similar in that all the trials used CT angiography to identify patients with large artery proximal occlusions. They differed in that ESCAPE, EXTEND-IA and SWIFTPRIME used multimodal CT imaging (multiphase CTA in ESCAPE and CTP in EXTEND-IA and SWIFTPRIME) to select patients. These latter three trials demonstrated the largest treatment effect size, presumably by excluding more patients who would have poor outcomes despite treatment.

Both ESCAPE and SWIFTPRIME developed a strong focus on the speed of treatment. The key messages from the sum of these trials are as follows:

1) Patients must be selected with CTA to identify a target lesions – a proximal artery occlusion.
2) Patients with very poor collaterals or very large core infarction should be excluded because they will not substantially benefit. The definition of the best imaging paradigm for patient selection continues to be debated.
3) Treatment must be applied very quickly. The target interval times in ESCAPE were: CT-to-groin puncture < 60 minutes; and CT-to-reperfusion < 90 minutes. The ESCAPE trial

demonstrated that median times below these thresholds were possible. However, the 90th percentile times were well above these thresholds[3].

4) High rates of reperfusion must be achieved rapidly and safely.

The strong evidence that endovascular therapy is effective in improving neurological outcome in patients with large vessel arterial occlusions now provides a human paradigm of the ischemia-reperfusion model that simulates animal models of ischemia-reperfusion that are widely used in preclinical stroke research and in which stroke therapies, especially neuroprotectants, have been evaluated. Until recently, the potential of endovascular recanalization to improve clinical outcome in past human trials was confounded by the failure of imaging selection, slow treatment paradigms and failure to fully reperfusion the brain. It is likely that such trials included patients in whom the brain was no longer salvageable, or in whom arterial occlusion was longstanding. Based on knowledge from preclinical animal studies, it is likely that permanent ischemia models are much more resistant to treatment. The results of the most recent endovascular trials now provide us with the opportunity to properly explore the concepts of neuroprotection and reperfusion injury and whether pharmacological or biological agents may allow incremental improvements in outcome for patients treated in this fashion. The human paradigm will no longer be confounded by a failure of reperfusion and the endovascular trials have successfully defined the target patient population with potentially salvageable brain. The use of NA-1 will be explored as an adjuvant agent for neuronal cytoprotection in the setting of AIS.

A key benefit of this approach is that NA-1 may result in a "freezing" of the core until reperfusion can be achieved[15]. This concept may extend to the application of treatment at distant sites to buy time until reperfusion therapy can be achieved. Further, there is evidence that cellular damage induced by ischemia continues even after reperfusion. This, so-called reperfusion injury, may be attenuated by adjuvant medical therapies such as NA-1.

4.8 Imaging as a Biomarker for Patient Selection

There is consensus in the stroke research community that a treatment benefit can only accrue if the treatment is given to a subject with salvageable tissue-at-risk. The theoretical construct used to define this tissue at risk, or "penumbra" using medical imaging comprises the difference between imaging tissue destined for infarction and tissue that is already irreversibly infarcted (the "mismatch hypothesis"). Whereas the best method for imaging "mismatch" remains controversial, in this study we are operationally defining "mismatch" using simple CT or MRI criteria that are rapidly implementable and are unambiguous. These criteria aim to enroll subject with large volumes of tissue-at-risk based on the finding of a large arterial occlusion while exclude those with large core infarctions, as the latter are unlikely to benefit from endovascular therapies[21].

Based on a synthesis of past studies, we have derived the following key clinical and imaging criteria for selecting subject for this study:

1) Moderate to severe ischemic stroke defined as >5 on the NIHSS.
2) No evidence of extensive infarction, defined as tissue hypodensity on NCCT scan on the basis of Alberta Stroke Program Early CT Scan (ASPECTS) scoring system, or as extensive apparent diffusion coefficient (ADC) restriction/DWI lesion volume (>100cc) on MRI.

3) A large arterial occlusion (carotid artery or proximal MCA) as defined by non-invasive imaging.
4) Evidence of adequate pial collateral filling.
5) Arterial occlusion amenable to safe and fast recanalization using endovascular means or combined intravenous thrombolysis plus endovascular treatment.

These criteria can be met using CT scanning and the data can be gathered and interpreted in <10 minutes. Using the ASPECTS, extensive infarction can be excluded. Using CTA detailed information can be gained on the location of occlusion and the burden of thrombus. Further, the CTA (from arch to vertex) can aid the interventionist in pre-planning navigation from the aortic arch to the thrombus. The pattern of collaterals is also discernible from CTA; patients with absent or very poor collaterals do not benefit from therapy, simply because it cannot be brought to bear in a timely enough fashion to prevent significant infarction. In ESCAPE[3], slightly more than half of patients were imaged using multi-phase CTA. Single-phase CTA and dynamic/multiphase CTA were used to select patients in the remaining cases. Many sites had access to CTP imaging but did not use it for patient selection. However, the pattern of collateral circulation can be assessed using CTP imaging.

Thus, a fast image acquisition "good scan (small core), proximal occlusion, adequate collaterals" model of patient selection is being adopted. This group of patients has shown a large treatment effect of endovascular therapy. This group of patients represents the near ideal "ischemia-reperfusion" paradigm. Thus we propose that it is the best subset of stroke patients in which to test the benefits of neuroprotection.

5 TRIAL OBJECTIVES

5.1 Primary Objective

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

5.2 Secondary Objectives

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

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

5.3 Tertiary Objectives

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

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

5.4 Leading Safety Objectives

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

6 TRIAL DESIGN

This is a Phase 3 randomized, multicentre, blinded, placebo-controlled, parallel group, single-dose design. Subjects harboring an acute stroke and who are selected for endovascular revascularization in accordance with local institutional practices and who harbor a small established infarct core and with good collateral circulation will be given a single, 2.6 mg/kg (up to a maximum dose of 270 mg) intravenous dose of NA-1 or placebo as soon as they are deemed to have met the enrollment criteria and with the intention of starting administration within 30 minutes of randomization.

A total of 1120 subjects aged 18 years and older will be enrolled into the study. All subjects will be followed for 90 days (or until death if prior to 90 days). Further details on the study procedures can be found in Table 2-1: Schedule of Assessments. The end of study is defined as the date that the last enrolled subject has completed their Day 90 visit.

6.1 Outcomes

6.1.1 Primary Efficacy Outcome

The primary efficacy outcome variable for the pivotal assessment of efficacy for regulatory submission purposes is the overall proportion of subjects experiencing a favorable functional outcome 90 days post-randomization, defined as 0 to 2 on the mRS. These subjects are defined to be responders.

6.1.2 Secondary Efficacy Outcomes

The key secondary efficacy outcome is the shift of one or more categories to reduced functional dependence analyzed across the whole distribution of scores on the mRS at Day 90 or the last rating.

Other secondary outcomes include:

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

6.1.3 Tertiary Outcomes

The following tertiary outcomes will be assessed descriptively:

1) Health-related quality of life, as measured by the EQ-5D-5L at Day 90 or the last rating.
2) Volume of stroke as measured by a) DWI and b) FLAIR at 24 Hour follow-up. If NCCT was done instead of MRI, Volume of stroke will be derived from hypodense areas.
3) Cognitive outcomes, as measured by the 15-item Boston Naming Test (BNT15), Sunnybrook Hemispatial Neglect Procedure (SNAP) and the Montreal Cognitive

Assessment (MoCA).

4) The proportion of subjects experiencing a favorable functional outcome 30 days post-randomization, defined as 0 to 2 on the mRS.

6.1.4 Safety Outcomes of Special Interest

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

6.1.4.1 Guidance on Definitions of Selected Safety Outcomes

Symptomatic intracranial hemorrhage (ICH) defined according to the Heidelberg classification, as a new ICH [intracerebral hemorrhage, SAH, intraventricular hemorrhage (IVH), subdural hematoma (SDH)] associated with clinical evidence of neurological worsening, in which, the hemorrhage is judged to be the most important cause of the neurological worsening. Clinical worsening will be guided by the NIHSS score of a minimum of two or more points different from baseline.

Major extracranial hemorrhage defined as life-threatening, resulting in hemodynamic compromise or hypovolemic shock, requiring inotropic support or other means to maintain cardiac output, requiring blood transfusion of more than two units of packed red blood cells, or associated with a fall in hemoglobin greater than or equal to 5 g/L.

Contrast media-induced nephropathy will be defined as a rise of the serum creatinine of ≥ 0.5 mg/dl (≥44 mmol/l) or a ≥25% increase from baseline pre-treatment level within 3 days after the endovascular procedure without an alternative etiology. Any creatinine measurements conducted as part of the protocol or standard of care in the first 3 days after the endovascular procedure in patients suspected by their treating physician of having a contrast nephropathy will be considered for this adjudication. .

Total radiation dose will be measured in mSv and recorded directly from the CT scanner. Dose-length product will be recorded directly from the CT scanner.

Malignant MCA infarction will be defined as any large infarction with mass effect observed on imaging where treatment (medical or surgical) is required for the treatment of mass effect.

Hemicraniectomy will be defined as that surgical procedure used to decompress the swollen hemisphere.

Vessel perforation will be defined at angiography by the operator and associated with sub-arachnoid hemorrhage.

Iatrogenic arterial dissection will be defined at angiography by the operator.

Arterial access site hematoma will be assessed as a complication of arterial access puncture and defined by clinical examination and anatomic imaging.

Retroperitoneal hematoma will be assessed as a complication of groin puncture and defined by imaging (ultrasound or CTA or MR).

Femoral neuropathy will be defined clinically and shall be considered related to the procedure if it is ipsilateral to the angiography puncture site and occurring with several weeks of the procedure.

6.2 Randomization and Blinding

Treatment will be assigned using 1:1 randomization (placebo:NA-1) centrally, using a web-based algorithm with treatment assignment allocated by web-based real-time interaction with the site. Randomization will be fully concealed by having both dynamic real-time allocation based upon random number generation and visually identical appearing NA-1 and placebo vials. All vials will have a unique vial number.

In order to minimize differences between the two treatment arms of the trial other than the investigational treatment, the protocol will restrict the use of multiple non-randomized intra-arterial therapies and stratify randomization by (a) use of intravenous thrombolysis with alteplase (yes/no) and (b) the declared first thrombectomy approach. The intended first thrombectomy approach will be restricted to the use of either a stent retriever or clot aspiration device. The intent to use any other endovascular device type or intra-arterial medications as the initial thrombectomy approach constitutes an exclusion criterion. Additionally, if intravenous thrombolysis is planned for the subject, the intent to use any intravenous thrombolytic other than alteplase is an exclusion criterion.

Thus, randomization will be stratified by declared first thrombectomy approach which will be dichotomized as stent retriever vs aspiration device, and by intravenous alteplase use (yes/no). Within each stratum, a randomized minimization procedure will occur on 6 baseline prognostic variables. Subject data required for randomization are age, sex, baseline NIHSS scores, baseline ASPECTs score, occlusion location and site.

The purpose of stratification is to provide increased certainty that equal or near-equal proportion of patients in each stratum will receive NA-1 or placebo.

The purpose of minimization on the variables of age, sex, baseline NIHSS score, baseline ASPECTS score, occlusion location (MCA or ICA), and site, which are important prognostic variables impacting the 90 day outcome, is to balance these variables across treatments to ensure a reduced chance that any observed effect size of NA-1 vs. placebo is confounded by these known important prognostic variables. This randomized minimization method from Zhao et al, called the minimal sufficient balance method[23], will be used to ensure that the subjects entered into the trial will be balanced between control and active treatment arms. Further details of the minimization procedure are provided in Appendix 3 – Randomization Details.

The time of randomization is defined as the time randomization occurred on the central server and this time is considered time zero for the study. Study drug will be infused immediately after randomization with the intention of starting administration within 30 minutes of randomization. Study drug should be administered within 60 minutes of the NCCT.

All subjects that are randomized will be accounted for in the trial database and followed rigorously for the primary and key secondary outcomes to minimize issues of imputation/missing data. The randomization number and time will be automatically printed from the randomization website and it will be transmitted to the central database to create the case in the electronic case report form (e-CRF). The randomization date, time, the two stratification variables, tPA use, first declared device and the six key randomization variables, age, sex, baseline NIHSS, baseline ASPECTS, occlusion location and site will not be editable once the subject is randomized. Thus, once a subject is entered into the randomization website, that subject's e-CRF will be auto-created and ready for data entry.

The automated system will inform site staff when re-stocking of the refrigerator is required and will specify the vial numbers to be restocked. This will ensure that a blinded investigator cannot match a vial that was just given to a subject to a single re-stocked vial and adds to the assurance of the study blind. Re-stocking will take place within one business day per the local participating site's working practice.

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

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

The person responsible for the study drug labelling will be unblinded, as will the independent statistical group preparing the reports for the IDMC. The person responsible for the data management group, who manages the programming of the randomization system will be unblinded. This individual will be the contact person in the event that unblinding is necessary (Section 6.3). This individual will not participate in data management and will only communicate unblinded data as set out below when contacted by the medical monitor.

6.3 Procedure for Breaking the Randomization Code

To maintain the overall quality and legitimacy of the clinical trial, code breaks should occur only in exceptional circumstances when knowledge of the actual treatment is absolutely essential for further management of the patient to ensure their safety and well-being. The Investigator is requested to maintain the blind as far as possible. If at all possible, the actual treatment allocation should not be disclosed to the subject and/or other site personnel unless, in the judgement of the investigator, this information is required for the subject's safety. The actual treatment allocation must not be disclosed to study personnel on site not involved in the subject's medical care, to monitors or the sponsor. The investigators have the final decision and unilateral right for unblinding.

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

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

In order to fulfill expedited regulatory reporting requirements, the Sponsor may be required to unblind the subject if the SAE meets the criteria for reporting to health authorities. The Medical Monitor or Drug Safety Manager will initiate the request that the subject's treatment group be

unblinded. In this case, the code will be broken only for the subject(s) in question. The information resulting from code-breaking (i.e., the subject's dose assignment) will not be communicated to either the Investigator or any other clinical team member of the Sponsor.

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

6.4 Treatment

6.4.1 Dosage Form, Accountability and Labeling

NA-1 was formulated at 20 mg/ml under current Good Manufacturing Practices by The University of Iowa College of Pharmacy in 50 mM sodium phosphate buffer with 0.45% sodium chloride (NaCl), potential hydrogen (pH) 7.0. This formulation was dispensed aseptically into 30 mL vials with snap cap lids (13.5 mL) intended for a single-dose intravenous injection of NA-1.

Placebo consists of the same buffer used for NA-1 with slightly higher NaCl content to adjust for equivalence of osmolality between drug product and placebo. It is supplied in identical vials containing 13.5 mL of 50 mM sodium phosphate pH 7.0 (0.55% NaCl).

Formulated NA-1 and placebo will be delivered in sterile, single-use individually labeled vials (serum vials) each with a unique five-digit identification number each with a unique five-digit identification number, and will be stored at the clinical site at 2 to 8°C in a secure location with restricted access.

Records will be made of the receipt and dispensing of the study drugs supplied. Documentation for each study drug 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/Five-digit Code
4) Expiry and/or Manufacturing and/or retest date
5) Dispensing information.

Drug vials will be labeled in accordance with applicable regulatory requirements.

6.4.2 Disposition of Study Drug Supplies

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

6.5 Study Drug Monitoring

At each applicable monitoring visit, the monitor will verify that:

- Study drug vials are appropriately stored in the refrigerator at 2 to 8 °C in a secure location, and that they have the correct expiry date
- CT area refrigerators are being re-stocked in a timely fashion
- 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

6.6 Case Report Forms

All source documentation will be reviewed for compliance with GCP. Case report forms (CRF) for the study are electronic.

6.7 Process, Timing and Quality Assurance Metrics

To ensure that the process and quality of key activities related to randomization, drug dosing and thrombectomy procedures and patient follow up are in place, process (including timing) and quality assurance metrics will be followed on an ongoing basis throughout the study by reviewing data collected in the CRF. The metrics that will be followed include:

1) Time from randomization to start of study drug administration (<30 min)
2) Time of Drug infusion start to drug infusion completion (10 ± 1 minutes)
3) Time of stroke onset to time of study drug infusion start
4) Time of stroke onset to reperfusion
5) Time of start of study drug administration to reperfusion
6) NCCT-to-study drug initiation time (<60 min)
7) NCCT-to-groin puncture time ("picture-to-puncture") (<60 min)
8) NCCT-to-recanalization time ("picture-to-reperfusion") (<90 min)
9) Rate of aspiration pneumonia
10) Rate of use of general anesthesia
11) Rate of symptomatic deep venous thrombosis/pulmonary embolism
12) Proportion of subject with any SAE per site
13) Fidelity of primary thrombectomy device intended for use versus primary thrombectomy device actually used.

7 SELECTION AND WITHDRAWAL OF SUBJECTS

7.1 Study Enrolment Process

Subjects will be identified using usual standard of care screening methods at the acute stroke hospital. This will include screening by neurology residents, fellows, nurse practitioners, physician assistants or faculty physicians.

Sites will only be selected to participate in the study if they have established mechanisms for screening this population of subject. This includes standard of care use of NCCT and CTA.

All subjects will undergo an acute clinical assessment, blood laboratory assessment and baseline brain imaging. For the standardization of the imaging criteria for patient selection across sites, all sites will perform a NCCT brain and CTA to determine patient eligibility. The NCCT head will be assessed using ASPECTS (Alberta Stroke Program Early CT Score) prior to randomization. Patients with an ASPECTS score of 5 or greater may be included in the study. ASPECTS is a reliable and reproducible assessment tool with low inter- and intra-observer variability[22] and is used to standardize the imaging criteria used for patient selection. The CTA will be also assessed for collateral status using a collaterals scoring system similar to that used for patient selection in the ESCAPE trial. Instructions for the determination of the ASPECTS score and collaterals scoring on CTA are provided in Appendix 2: Derivation of ASPECTS on NCCT and Collaterals Scoring on CTA and additional details can be found at www.aspectsinstroke.com.

From the perspective of imaging to recanalization and other interval times, the first slice of the baseline NCCT is when the timing begins.

If CTP is done routinely at the site, NCCT and CTA must still be used to determine inclusion criteria. It is mandated that a CTP cannot be performed before the CTA.

In order to track the potential for enrolment, each enrolling site will provide the total number of patients admitted to that site with the diagnosis of AIS. Individual patient screening logs will not be required.

If the subject remains eligible after completion of routine screening, the patient will be consented (as required) and enrolled into the study. A patient who consents but is not randomized will be considered a screen failure. A subject is considered randomized the moment the randomization process is completed on-line. This is time "0" for the study. Subjects who are randomized but do not receive study drug will still be followed through the 90-day study period.

7.2 Inclusion Criteria

1) Acute ischemic stroke (AIS) for immediate endovascular treatment.
2) Age 18 or greater.
3) Onset (last-seen-well) time to randomization time within 12 hours.
4) Disabling stroke defined as a baseline NIHSS > 5 at the time of randomization.
5) Pre-stroke (24 hours prior to stroke onset) independent functional status in activities of daily living with modified Barthel Index (BI) > 90 (95 or 100). Patient must be living in their own home, apartment or seniors lodge where no nursing care is required.

6) Confirmed symptomatic intracranial occlusion, based on multiphase or dynamic CTA, at one or more of the following locations: Intracranial, carotid T/L, M1 MCA. Anterior temporal artery is not considered an M2. Functionally, when defining the M1 MCA, the bulk of the MCA territory must be ischemic.
7) Non-contrast computed tomography (NCCT) and CTA* for trial eligibility performed or repeated at ESCAPE-NA1 stroke centre with endovascular suite on-site.
8) Endovascular treatment with declared first endovascular approach as either stent retriever or clot aspiration device, and intended to be initiated (arterial access) within 60 minutes of baseline/qualifying NCCT with target baseline/qualifying NCCT to first recanalization of 90 minutes. Study drug intended to be administered within 60 minutes of the baseline/qualifying NCCT.
9) Signed informed consent from subject or legally authorized representative or, if required to enable inclusion by applicable national laws and regulations and the applicable independent review boards/Ethics Committee requirements for obtaining consent, from the investigator after consultation with an independent physician who is not otherwise participating in the trial.

*As per the List of Abbreviations (Section 1.2), all references to CTA indicate a multiphase or dynamic CTA.

7.3 Exclusion Criteria

1) Evidence of a large core of established infarction defined as ASPECTS 0-4.
2) Evidence of absence of collateral circulation on CTA (Collateral score of 0 or 1).
3) Intent to use any endovascular device other than a stent retriever or clot aspiration device or intra-arterial medications as the initial thrombectomy approach.
4) Intent to use any intravenous thrombolytic other than alteplase if intravenous thrombolysis is planned.
5) No femoral pulses, very difficult endovascular access or extreme tortuosity of the great vessels that is predicted to result in an inability to timely deliver endovascular therapy. Direct common carotid or radial/brachial/axillary access is permissible.
6) Estimated or known weight > 120 kg or < 45 kg.
7) Pregnancy; if a woman is of childbearing potential a urine or serum β-hCG test is positive, or breastfeeding.
8) Severe contrast allergy or absolute contraindication to iodinated contrast preventing endovascular intervention including any contraindications listed in the prescribing information approved by local authorities (e.g., patients with decompensated heart failure as a contraindication for the use of VISIPAQUE™ 270 in Germany).
9) Clinical history, past imaging or clinical judgment suggests that the intracranial occlusion is chronic or there is suspected intracranial dissection such that there is a predicted lack of success with endovascular intervention.

10) Prior enrolment in the ESCAPE-NA1 trial or prior receipt of NA-1 for any reason.

11) Severe known renal impairment defined as requiring dialysis (hemo- or peritoneal) or if known creatinine clearance < 29 mL/min.

12) Patient has a severe or fatal comorbid illness that will prevent improvement or follow-up.

13) Patient cannot complete follow-up treatment due to co-morbid non-fatal illness or they are a visitor to the city or any other known reason that follow-up would be impossible (e.g. incarcerated in a federal prison).

14) Participation in another clinical trial investigating a drug, medical device, or a medical procedure in the 30 days preceding study inclusion.

This population is expected to consist of subject very similar to those treated in the ESCAPE trial.[3]

8 TREATMENT OF SUBJECTS

8.1 Study Interventions

Patients who are deemed to be candidates for endovascular intervention in accordance with the inclusion/exclusion criteria will be randomized to receive NA-1 or placebo.

8.1.1 Administration of Study Drug

8.1.1.1 Timing of Dose Administration

The enrolling specialist will determine if the patient is a candidate for endovascular reperfusion based in the study inclusion/exclusion, consent has been obtained and randomization allocation has occurred.

At the time that a patient is deemed to be eligible for endovascular recanalization, the research coordinator (or delegate) will log into the central randomization website, enter key variables, and the website will inform which of the vials in the refrigerator should be used for that subject based on the current balance in the trial assignment of the baseline characteristics of all subjects randomized to date. The research coordinator (or delegate) will then select that single vial of study drug from the refrigerator and prepare it for administration.

Dose timing shall start at the time of drip onset. Study drug is intended to be administered within 60 minutes of the NCCT and within 30 minutes of randomization. The time of dosing onset and the time at which the endovascular angiographic procedure began (insertion of an angiogram sheath into the femoral (or other relevant) artery at the start of the procedure) will be recorded on the CRF.

8.1.1.2 Dose Administration

Dosing will be carried out by, or under the supervision of, the trial physician who is supervising the care of the subject for the planned or ongoing endovascular reperfusion procedure.

A syringe for each individual subject dosing will be prepared by calculating the volume to draw from the vial as follows: for subjects weighing less than 105 kg: (2.6 mg/kg x subject weight in kg)/(20 mg/ml). This will determine the number of mL to pull up into the syringe. For subject weighing 105-120 kg the full volume of one vial (13.5mL, equivalent to 270 mg of NA-1) will be used.

The syringe containing study drug will be injected into the intravenous port of a 50 or 100 mL drip bag of 0.9% normal saline that has been labeled with the randomization number and subject's age. The bag will be mixed by squeezing and inverting the bag several times. After dilution into the drip bag, the drug is stable for up to 5 hours, however it should be infused into the subject as soon as practical with a target randomization to treatment time of 30 minutes or less.

An appropriate sized syringe must be used, such that this volume is between 20% and 100% of the delineated markers on the syringe. Once a volume of drug has been drawn into the syringe, the vial will be marked as "used" and placed into a separate vial storage area.

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

After the dose administration, a minimum of 10 mL of saline will be administered using the infusion pump, to flush any remaining medication left within the intravenous tubing. This method of dose administration is identical to that previously conducted in the Phase 2 ENACT trial[11]. In the event that a subject is concomitantly receiving alteplase therapy, dose administration of NA-1 will be conducted through a separate intravenous catheter inserted into an extremity not containing the alteplase line. If alteplase therapy has been completed, the same intravenous catheter may be used after it has been flushed with saline.

8.2 Interventions

8.2.1 Endovascular Intervention

The neuro-interventionalist will pre-declare, prior to randomization, their preferred first endovascular device type to be used in the procedure, defined as either a 'stent retriever thrombectomy' or 'aspiration thrombectomy'. Any currently available and approved/cleared device may be used for this purpose. The neuro-interventionist should adhere to using this declared device when making their first approach. If the first approach fails, the neuro-interventionalist may use their best medical judgment to complete the procedure in the patient's best interest. The use of a balloon guide catheter is recommended with the stent retriever approach.

Any use of an intra-arterial medication such as a thrombolytic agent (eg. alteplase) or antiplatelet agent is known to be uncommon and will be recorded in the concomitant medications list. Permanent stents should not be left in the intracranial circulation or in the internal carotid artery, except under exceptional circumstances dictated by the needs of acute patient care.

Guidance for recommended approaches to endovascular revascularization are provided in Appendix 1 – Guidance on Imaging Selection and Endovascular Treatment.

8.2.1.1 Acute Carotid Artery Stenting

It is recommended that carotid artery stenting should not be undertaken acutely in order to avoid the need for dual antiplatelet agents. It is strongly recommended that neuro-interventionists tackle the intracranial occlusion primarily and only then consider the extracranial carotid artery. In some circumstances, carotid angioplasty, without stenting may be required with a planned return to the neuro-intervention suite 2-3 days later for definitive carotid management with stenting. It is recognized that carotid artery stenting may still be required in some circumstances. The use of carotid artery intervention (angioplasty with or without stenting) will be monitored and specific data collected.

8.3 Guidelines-based Medical/Stroke Unit Care

All acute stroke subjects should receive the best standard of care according to national guidelines. The model will be the Canadian best practices guidelines for acute stroke care. These are very

similar to the guidelines of the American Stroke Association and the European Stroke Organization. All subjects are expected to be admitted to hospital as part of routine standard of care.

It is expected that all subjects will undergo a routine work-up for the mechanism of their stroke and be treated appropriately and definitively. This is critically important because subjects who have an excellent early recovery are at the highest risk of early recurrent stroke.[24] We wish to prevent recurrent stroke from confounding the 30-day and 90-day clinical outcome such that subject who are well at discharge remain that way for the duration of the 90-day follow-up period.

We expect the following preventive care. Relevant subject with atrial fibrillation should be anti-coagulated. Patients with symptomatic carotid artery stenosis should undergo carotid revascularization early and definitely within two weeks of stroke onset.[25] Risk factors, including hypertension, elevated cholesterol, diabetes mellitus, tobacco smoking, should be treated appropriately and aggressively according to current standards of care.

We expect subjects to receive adequate hydration to prevent renal complications. Patients will receive intravenous radio-contrast media for CTA for endovascular intervention. While these medications are generally extremely safe in stroke patients,[26] simple hydration can prevent renal complications, particularly among patients with baseline borderline renal function and among those with diabetes mellitus. Further, patients with ischemic stroke are generally slightly hypovolemic at baseline. We recommend use of intravenous normal saline (0.9% saline) infusion at 1.5 – 2.0 cc/kg/h until the subject is eating and drinking safely and well. Therefore, for the typical subject this will mean intravenous normal saline at 75-125 cc/h overnight only. We do not recommend the use of bicarbonate solutions or N-acetyl-cysteine solutions for renal protection.[27,28] In general, the risk of nephrotoxicity from modern non-ionic, iso-osmolar radiocontrast media is extremely low.

For subjects that are disabled from their stroke and require a longer in-patient stay and/or rehabilitation, it is expected that they will receive standard stroke unit care to prevent complications. These include:

- Deep vein thrombosis (DVT) prophylaxis for subject who are bed-bound or primarily bed-bound
- Swallowing assessments and prevention of aspiration pneumonia
- Early mobilization and physiotherapy to prevent skin breakdown, pneumonia, DVT/pulmonary embolism beginning 24 hours after randomization
- Early diagnosis and treatment of fever

All subjects are expected to receive expert stroke unit care and then rehabilitation according to their clinical need.

In brief, it is expected that:

1) Patients who are appropriate candidates for intravenous thrombolytic therapy within 4.5 hours of defined stroke onset to get rapidly administered intravenous alteplase therapy.
2) All subjects should be managed urgently with attention to physiology. Airway, breathing and circulation (ABC) first. Fluids – usually normal saline - should be administered, as

most patients are slightly hypovolemic. BP should not be aggressively treated other than to guidelines to allow thrombolytic therapy.

3) All subjects should be managed on a stroke unit which should include early mobilization, early rehabilitation, DVT prophylaxis, swallowing assessment and prevention of aspiration pneumonia, nasogastric (NG) feeding as appropriate and delayed (up to four weeks) placement of percutaneous endoscopic gastrostomy (PEG) tubes in the uncommon instance that these are needed.
4) All subjects should receive ASA therapy (minimally) within the first 48 hours of stroke but after a day plus one brain image showing no evidence of major hemorrhage.
5) Patients should not receive intravenous unfractionated heparin.
6) All subjects should be thoroughly investigated to sort out the mechanism of stroke and appropriate stroke prevention strategies implemented. There is little use in working very hard on a technical and aggressive approach to acute stroke if it is to be undone on day +10 with a recurrent embolus because a subject has not been effectively treated for a preventable cause of stroke.
7) All subjects should have relevant and appropriate stroke rehabilitation therapy including physical, occupational and speech therapy.

In summary, it is expected that all subject should have excellent, guideline based stroke care through the full 90 days.

8.4 Speed of Intervention

A key principle of the neuroprotective and endovascular intervention is that it must be both fast and safe. The target NCCT to revascularization time is the 90th percentile < 90 minutes and median at 75 minutes or less. Similarly, an interim measure will be the NCCT to groin puncture time at 90th percentile of <60 minutes and median at <45 minutes. This will be measured from the time of the first slice of the baseline/qualifying NCCT head done at the ESCAPE-NA1 centre. The target complication rate is less than 1%. The quality of intervention will be ensured by hand-selection of sites and only be approved by the executive committee after a site visit. All sites will be selected from the ESCAPE trialists. New sites must submit evidence within the two years prior to commencement of the trial that they can meet the 90-minute target of CT-to-recanalization time. For sites that participated in the ESCAPE trial, their trial data will constitute proof of capability of treatment. A principle of the evaluation of neuroprotection by NA-1 is that it should be implementable without significantly delaying the subject's access to reperfusion.

A key and critical component of the trial will be an ongoing quality assurance program to ensure that sites can meet these targets for endovascular intervention. Training will be undertaken at the sites and continued on a quarterly basis. Monitoring of interval times will be collated and provided to sites on a quarterly basis so that regular feedback might induce appropriately fast treatment processes. Sites that fail to meet these objectives in the trial will be dropped from the trial.

The sites will be assisted to achieve these targets.

8.5 Consent Process

Signed informed consent will be obtained prior to any protocol-specific procedures from the subject or legally authorized representative or, if required to enable inclusion by applicable

national laws and regulations and the applicable independent review boards/Ethics Committee requirements for obtaining consent, from the investigator after consultation with an independent physician who is not otherwise participating in the trial.

If the original process involved anyone other than the subject , and if required by local standards, consent will be sought for the remaining procedures from the subject once they are deemed to have regained capacity.

8.6 Baseline Clinical and Laboratory Evaluations

At Baseline, all subjects will undergo a routine neurological and clinical assessment (including history, examination and vital signs), the NIHSS and pre-stroke BI.

Emergency blood work will be drawn including: CBC (hemoglobin, platelets and hematocrit), electrolytes (sodium, potassium, chloride), INR, aPTT, serum creatinine and serum glucose. The study will use and collect local laboratory results and will not use a central lab. The results of this blood work are not required prior to randomization.

If the subject is female and is of childbearing potential a pregnancy test (urine point-of-care pregnancy test) must be completed and the result must be negative; this is the only mandatory laboratory test prior to randomization. Other laboratory or point-of-care testing may be performed at the discretion of the attending physicians and team.

An ECG should be completed at baseline. If the treating physician deems that a baseline ECG impedes the patient's access to timely care, the ECG must be performed within 6 hours of randomization.

All prior medications taken within three days of treatment initiation will be recorded on the e-CRF.

8.7 Immunogenicity Substudy

Plasma samples for immunogenicity analysis will be collected at Day 1 and Day 30 from up to 250 subjects enrolled at a subset of sites in Canada and the US.

8.7.1 Immunogenicity Sample Collection

At Day 1 (24 hours post dose – a time when no significant quantities of NA-1 remain in the blood) and Day 30 a 5 mL sample of blood will be collected in tubes provided by the Sponsor. Sample collection method should be the least invasive to the subject and may be combined with routine blood sampling.

The samples will be handled per guidelines/instructions provided by the sponsor. These tubes are labeled with adhesive labels that identify the study code, site number, the subject's number, and the sample Day. A minimum of 1.0 mL of plasma will be transferred into each cryovial.

The date and time for each step (blood draw, storage, centrifugation, aliquoting, and freezing) and the number of aliquots obtained must be documented in the source document.

8.7.2 Immunogenicity Sample Storage and Shipment

During storage, plasma tubes are to be kept in a freezer at -10°C or colder. The temperature will be monitored and documented for the duration of any sample storage.

Plasma will periodically be shipped frozen to the sponsor. Further shipping instructions will be provided by the Sponsor.

8.8 Pharmacokinetics Substudy

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

8.8.1 PK Sample Collection

A total of six 2 mL blood samples for PK assessment will be drawn during the Baseline Visit. Time (T) =0 being the initiation of dosing of study drug or placebo. Samples will be drawn at the following timepoints:

- Sample 1: post randomization & prior to start of study drug administration (Time=0 min)
- Sample 2: 10 minutes from start of study drug administration (Time = 10 min)
- Sample 3: 20 minutes from start of study drug administration (Time = 20 min)
- Sample 4: 30 minutes from start of study drug administration (Time = 30 min)
- Sample 5: 60 minutes from start of study drug administration (Time = 60 min)
- Sample 6: 120 minutes from start of study drug administration (Time = 120 min)

At each timepoint a 2 mL sample of blood will be collected in tubes provided by the Sponsor. Sample collection method should be the least invasive to the subject and may be combined with routine blood sampling.

The samples will be handled per guidelines/instructions provided by the sponsor. These tubes are labeled with adhesive labels that identify the study code, site number, the subject's number, and the sample date and time. A minimum of 1.0 mL of plasma will be transferred into each cryovial.

The date and time for each step (blood draw, storage, centrifugation, aliquoting, and freezing) and the number of aliquots obtained must be documented in the source document.

8.8.2 PK Sample Storage and Shipment

During storage, PK tubes will be kept in a freezer at -10°C or colder. The temperature will be monitored and documented for the duration of any sample storage. PK samples will be stored in a separate cryobox from immunogenicity samples. All samples taken from the same subject should be stored in the same cryobox.

PK samples will be periodically shipped frozen to the Sponsor. Further shipping instructions will be provided by the Sponsor prior to study start up.

8.9 Brain and Neurovascular Imaging

All subjects will initially undergo rapid NCCT and CTA imaging. Routine NCCT and CTA imaging guidance and minimal acceptable criteria for image quality are provided in Appendix 2: Derivation of ASPECTS on NCCT and Collaterals Scoring on CTA. Sites that did not participate in the ESCAPE trial will submit sample images to the core lab for quality assessment.

The use of a dynamic CTA or multiphase CTA data acquisition protocol is mandatory for ESCAPE-NA1. This is detailed in Appendix 2: Derivation of ASPECTS score on NCCT and Collaterals Scoring on CTA.

Baseline NCCT and CTA may be completed at a hospital affiliated with the ESCAPE-NA1 stroke centre, only if the baseline NCCT to drug delivery time < 60 minutes and the appropriate NCCT and multiphase/dynamic CTA are completed at the affiliated hospital provided that the imaging studies are available in the same medical imaging network.

Imaging at follow-up (24h) which will be used to assess infarct volumes, will be conducted using MR.

Thus, imaging and imaging assessments will be fully standardized for all assessments.

For all interval times assessed from imaging, the time zero will be the first slice of the NCCT scan. The purpose of this approach is to include imaging time and post-processing time in to any time metric.

8.10 Study Drug Administration

Dosing will begin as soon as the enrolling specialist has indicated that the subject is a candidate for endovascular reperfusion based in the study inclusion/exclusion, consent has been obtained and randomization allocation has occurred. See Section 8.1 for additional details.

8.11 Evaluations Following Randomization

Vital signs will be completed pre- and post-dose and at 24 and 48 Hours, and Day 5. An ECG will be completed at 24 ± 12 Hours. Blood tests will be conducted at 24 ± 12 Hours including: CBC (hemoglobin, platelets and hematocrit), electrolytes (sodium, potassium, chloride), INR, aPTT, serum creatinine and serum glucose.

All subjects will undergo a follow-up brain MRI [including a minimum of axial DWI, gradient-echo (GRE), FLAIR] at 24 ± 12 Hours from the time of randomization. The 24-hour MR is considered a standard of care imaging procedure; if MR is unavailable, then NCCT is allowed.

All subjects will have a mRS scale scores evaluated at Day 5 or discharge, Day 30, and Day 90. Additionally, a pre-morbid (baseline) mRS will be collected and recorded on the 24h CRF page. All subjects will also undergo a NIHSS assessment at 2 Hours (end of the angiogram procedure), 24 and 48 Hours, and Day 5 or discharge, 30, and 90. The BI will be completed at Day 30 and 90 days. The EQ-5D-5L will be completed at Day 30 and 90 days.

Mortality and discharge disposition will be assessed at Day 5 or discharge. Mortality will also be assessed at Day 30 and 90. Then BNT, SNAP and MoCA will be conducted at Day 90.

SAEs will be collected through to Day 90. AEs and concomitant medications will be collected to Day 30. All time windows are calculated from the time of randomization (time '0').

8.12 Imaging

The baseline NCCT and CTA and all brain imaging conducted within the first 48 hours thereafter will be rendered anonymous and sent to the ESCAPE-NA-1 core lab (Calgary) for central adjudication. The 24 hour MR (and where MR is unavailable, CT) will be used to assess 24 Hour infarct volume, an exploratory tertiary outcome. Other brain imaging will be sent only if requested by the adjudication committee.

The core imaging lab staff will review all imaging for the acute stroke intervention period as per an Imaging Adjudication Charter in order to ensure adherence to the imaging guidelines and enrollment criteria, to determine reperfusion rates and quality of the intervention, and safety. Infarct volume determinations (an exploratory outcome) will not be conducted until after database lock.

8.13 Criteria for Discontinuation from the Study

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

- 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 legally authorized representative (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 e-CRF.

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

A subject may not withdraw use of his or her data that have already been collected, this is in alignment with the FDA guidance document FDA 21 CFR UCM126489, Data Retention When Subjects Withdraw from FDA-Regulated Clinical Trials[29] 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".

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 research coordinator to bring the subject in for a clinic visit for follow-up and this process will be documented.

9 ASSESSMENT OF EFFICACY

For the assessment scales listed below, sites and local investigators will be responsible for obtaining certification prior to conducting any assessments.

9.1 The Modified Rankin Scale

The primary endpoint used in this trial will be global disability, as measured by the mRS, at Day 90. The mRS is a valid and reliable clinician-reported measure of global disability that has been widely applied for evaluating recovery from stroke. It is a scale used to measure functional recovery (the degree of disability or dependence in daily activities) of people who have suffered a stroke[30,31]. mRS scores range from 0 to 6, with 0 indicating no residual symptoms; 5 indicating bedbound, requiring constant care; and 6 indicating death.

The mRS will be obtained at Day 5 (or discharge), Day 30 and 90. Premorbid mRS status will also be obtained retrospectively and reported on the 24h CRF page. The mRS will only be scored by those trained and certified in the use of this scale.

9.2 The National Institutes of Health Stroke Scale

The NIHSS is a standardized neurological examination score that is a valid and reliable measure of disability and recovery after acute stroke[32]. Scores range from 0 to 42, with higher scores indicating increasing severity. The scale includes measures of level of consciousness, extra ocular movements, motor and sensory tests, coordination, language and speech evaluations. The NIHSS will be administered at Baseline, Post-EVT (2 Hours), at 24 and 48 Hours, Day 5 or discharge, 30 and 90. The NIHSS will only be scored by those trained and certified in the use of this scale.

9.3 Barthel Index

The BI is an index of functional independence[33] that is a valid measure of activities of daily living when employed in stroke trials[34]. Modified BI scores range from 0 to 100, with higher scores indicating greater independence in activities of daily living and mobility. The BI will be scored at Baseline (pre-morbid), on Day 30 and 90 by those trained in the use of this scale. Note that the original Barthel Index was a scale from 0-20. The modified Barthel index simply multiplies the original scale by 5.

9.4 Mortality Rate

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

9.5 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[35]. Each dimension has five response categories corresponding to: no problems, slight, moderate, severe and extreme problems[36]. The instrument is designed for self-completion, 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 and 90 by those trained in the use of this scale.

9.6 Volume of Strokes

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

9.7 Cognitive Outcomes

The BNT15 is a widely used neuropsychological assessment tool to measure confrontational word retrieval in individuals with aphasia or other language disturbance caused by various neurological disorders.[37]

The SNAP is a short bedside battery for visuoconstructive hemispatial neglect shown to be a highly reliable and valid instruments with good internal consistency in stroke subject.[38]

The MoCA (www.mocatest.org) has been found to be a feasible global cognitive screening tool in stroke trials[39]. It has been found to be sensitive in detecting post-stroke cognitive impairment; raw scores using the published cut-off score of 26 showed good sensitivity but moderate specificity. Montreal Cognitive Assessment scores adjusted for age and education provide both good sensitivity and very good specificity.[40]

The BNT15 (short form)[41], SNAP and MoCA will be administered at Day 90 by those trained in the use of these scales. Assessments will only be conducted if the tool/scale is available in the local country language. If the assessment is not conducted for language reasons the outcome will not be measured for those subjects.

9.8 Pharmacokinetics Assessment

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

- **AUC_{0-t}:** Area under the concentration-time curve from time zero to time of last measurable concentration
- **AUC_{0-inf}:** Area under the concentration-time curve from time zero to infinity
- **C_{max}:** Maximum plasma concentration observed after dosing
- **T_{max}:** Time to occurrence of C_{max}
- **$t_{½}$:** Terminal elimination half-life

Samples with no detectable NA-1 will be excluded from analysis (placebo). A further analysis of the effect of administration of thrombolytic therapy (alteplase) on the pharmacokinetics of NA-1 will also be performed.

9.9 Additional Assessments

Two key concepts will also be explored in this trial:

1) The addition of a neuroprotectant to endovascular revascularization is feasible and can be done with a CT to Thrombolysis in Cerebral Infarction Score (TICI)3 flow in < 90 minutes.
2) Feasibility of recruitment rate, defined by the recruitment in this multisite trial.

10 ASSESSMENT OF SAFETY

10.1 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 unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

Therefore, an AE may be:

- A new illness
- The worsening of a concomitant illness
- An effect of vaccination, including the comparator
- A combination of the above

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.

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.

A serious adverse event (or reaction) is any untoward medical occurrence that at any dose:

1) Result in death
2) Are life-threatening
3) Require or prolong inpatient hospitalization
4) Result in persistent or significant disability/incapacity, or
5) Are a congenital/birth defect

A SAE can also be an important medical event that may not result in death, be life-threatening, or require hospitalization, but may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. For example, any new

diagnosis of cancer (made after study enrollment) is considered an important medical event. Because our primary safety outcomes for the trial are also SAEs by definition, they will be reported dually as SAEs and as outcomes. SAEs should be managed according to the best current standard of care.

All deaths occurring during the follow up to Day 90 will be reported as an SAE. When reporting a death, the event or condition that caused or contributed to the fatal outcome should be reported as a single medical concept.

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 occurring within 30 days of randomization and all SAEs will be reported in the CRF. Severity and relationship definitions are presented below.

Table 10-1: Definitions of AE-Related Terms

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	
Related	A clinical event, including laboratory test abnormality, where there is a “reasonable possibility” that the SAE was caused by the study drug, meaning that there is evidence or arguments to suggest a causal relationship.
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.
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.

Unexpected Adverse Drug Reaction

An adverse reaction, the nature or severity of which is not consistent with the applicable product information (e.g., Investigator's Brochure for an unapproved investigational medicinal product).

10.2 Clinical Management of Adverse Events

10.2.1 Early Study Drug Cessation

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

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

10.2.2 Treatment of Hypotension

If hypotension (systolic < 80 mmHg; or any level of decreased BP that the physician deems to be clinically relevant) is observed in a subject, the hospital physician will be instructed, at his/her discretion, to administer any medication that they deem to be required for the subject'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 NA-1. 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 subjects may be observed for spontaneous recovery.

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

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

All subjects will be monitored closely 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 e-CRF.

10.2.3 Increases in Histamine

NA-1 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, NA-1 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 NA-1 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, bronchoconstriction, 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 and establish airway first.
- Treat hypotension with crystalloid, colloid and/or vasopressors as needed (see Section 10.2.2).
- Consider treatment with antihistamine agents (diphenhydramine 50 mg IV, ranitidine 50 mg IV) and corticosteroids if the reaction is severe.
- Consider using subcutaneous or intravenous epinephrine.
- If bronchospasm or laryngospasm are important symptoms, consider treatment with inhaled racemic epinephrine.

10.2.4 Hyperglycemia

In the Phase 1 study, 9% of all subjects receiving any dose of NA-1 (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 NA-1 and placebo groups in blood glucose across time and at no time point 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 per the schedule of assessments as well as part of the standard-of-care. If hyperglycemia is noted, the physician will treat the subject on a case-by-case basis. As there is no known relationship between NA-1 administration and hyperglycemia (based on rat, dog, and human studies), there is no unique protocol for blood sugar management following NA-1 administration.

10.3 Identification of Adverse Events by the Investigator

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.

10.4 Reporting of Adverse Events

AEs should be reported as they occur on the e-CRF. 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.

10.5 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.

10.5.1 Reporting by the Investigator

All SAEs must be reported to the Sponsor within 24 hours of the local Investigator's first awareness of its occurrence. SAEs will be reviewed by the trial medical monitor.

The investigator will report the SAEs using the eSAE form in the eCRF, which will send an immediate alert to the Sponsor. If the eCRF system is not available, a paper SAE form should be directed within 24 hours to:

Pharmacovigilence, NoNO Inc.
redacted

10.5.2 Reporting SAEs to the Health Authorities and Ethics Committees

The Sponsor will inform the relevant health authorities of any reportable SAEs according to the local 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 the regulatory agencies (i.e., Health Canada, European Medicines Agency and FDA) 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 Sponsor or delegate will be responsible for reporting SUSARs to any Central Ethics Committees in compliance with local current legislation. The Investigators will be responsible for informing their local ethics committees of any reportable SAEs as per their local requirements.

10.6 Additional Safety Assessments

All pre and post dose vital signs, biochemistry, hematology laboratory tests and electrocardiograms must be reviewed by the investigator or qualified designee. Clinically significant results post dose will be recorded as an adverse event.

10.6.1 Vital Signs

Vital signs (BP, HR) will be taken in the supine position at Baseline (pre-dose), at the completion of drug infusion, 24 Hours, 48 Hours and Day 5/discharge. Temperature will be taken at baseline per standard of care and reported on the CRF if available. The Investigator will review the pre and post drug infusion vital signs.

If estimated weight was collected at randomization, the subject's actual weight will be measured in hospital using standard hospital scales (i.e., stand up or in-bed scales if the subject is not ambulatory), as soon as possible, but within five days. If actual weight cannot be measured for any reason (due to, for example severe illness or unavailability of in-bed scales at the site), weight will be determined by first asking the subject, second asking a family member or third by estimation.

10.6.2 Biochemistry and Hematology

Blood work will be taken at baseline and at 24 hours including: CBC (hemoglobin, platelets and hematocrit), electrolytes (sodium, potassium, chloride), INR, aPTT, serum creatinine and serum glucose. This is described in Sections 8.6. , 8.11 and the Schedule of Assessments.

If the subject is female and is of childbearing potential, a pregnancy test (urine or serum point-of-care pregnancy test) must be completed. Clinically significant laboratory findings from the 24-hour sample (post dose) will be recorded on the e-CRF as AEs.

10.7 12-Lead Electrocardiogram Monitoring.

A 12-lead ECG will be performed at Baseline or, if the treating physician deems that an ECG will impede the subject's access to care, within 6 hours of randomization, and at approximately 24 Hours. Clinically significant findings on the 24-hour ECG will be recorded on the e-CRF as AEs.

10.8 Concomitant Medications

Concomitant medications (Conmeds) will be collected from the time of randomization to the Day 30 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 30 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 30.

10.9 Safety and Data Review

In addition to the site investigator's review and IDMC reviews, on a regular basis the sponsor and external medical monitor will conduct a blinded safety and data review of the subject data collected to date in accordance with the Sponsor's Safety Management Plan.

10.10 Follow-up and Reporting of Pregnancies

Pregnancy is an exclusion criterion for enrolment in this study, but a subject could potentially 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 within one month of identifying a pregnancy case. 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).

11 STATISTICS

11.1 Sample Size Considerations

Assuming a 52% overall responder rate for the placebo group, there will be an approximately 80% power to detect an 8.7% absolute effect difference between response rate (proportion of responders, with Day 90 mRS in the range 0 to 2) with NA-1 and placebo, at alpha level 0.05, 2-sided with a planned sample size of 1076 evaluable subjects, randomized 1:1, per group, accounting for a single interim analysis when 600 subject have reached their primary endpoint assessments, with O'Brien-Fleming alpha-spending function (EaST® V6.3) stopping boundary for overwhelming efficacy and a non-binding 1% conditional power futility stopping boundary. The sample size will be inflated approximately 4% to N= 560 per group to account for loss-to-follow-up and drop-outs.

11.2 Analysis Populations

11.2.1 ITT Population

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

11.2.2 Per Protocol Population

The primary analysis will be repeated on the Per Protocol (PP) population, defined to be all subjects randomized and treated, with no major protocol deviations. This population will be determined via blinded review of protocol deviations at the end of the trial before database lock and unblinding.

Prior to unblinding, the imaging from each subject at the time of inclusion will be adjudicated to determine whether they have met the criteria for endovascular intervention, and hence for the trial. This will include review of baseline NCCT and CTA. Subjects who do not meet the imaging criteria outlined in the trial inclusion/exclusion criteria, will not be included in the Per Protocol (PP) population.

11.3 Analysis of Primary Efficacy Outcome

The primary efficacy outcome for the pivotal assessment of efficacy for regulatory purposes is the overall proportion subjects experiencing a favorable functional outcome 90-days post-randomization, defined as 0 to 2 on the mRS. These subjects are defined as responders. Subjects missing the day 90 primary assessment for any reason will be considered non-responders for the primary analysis (see Section 11.8). The primary hypothesis to be tested is that administration of NA-1 will result in an increase in the proportion of subjects with independent functioning on the mRS (as defined by a score of 0-2) at Day 90. The primary analysis will be a Wald test for treatment group difference in the primary outcome from a logistic regression adjusted for the 2 stratification variables (alteplase use, first declared thrombectomy device) and the 6 covariates used in the minimization: Age, sex, baseline NIHSS score, baseline ASPECTS score, occlusion

location and site. This will be conducted on the ITT population at the 2-sided 5% significance level overall (for the trial), adjusted for the interim analysis per the O'Brien-Fleming boundary spending function.

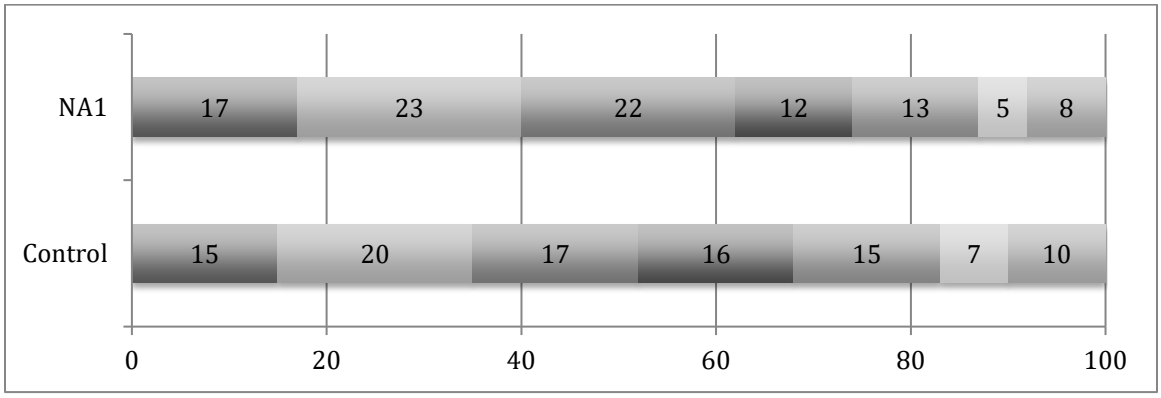
Three supportive analyses to the primary analysis will be conducted: (1) the primary analysis reapplied to the Per Protocol population; (2) a re-randomization analysis to demonstrate that minimization did not bias the primary endpoint analysis and (3) The primary analysis will be repeated on the PP population using actual first endovascular approach (instead of declared approach). Details of these are provided in the Statistical Analysis Plan.

11.4 Key Secondary Outcome Analysis

The study will also test the secondary hypothesis that among randomized subject, those who are treated with NA-1 will show shift in the distribution of scores on the mRS scale at 90 days, assuming that categories 5 and 6 (bedbound with severe disability, and death) are collapsed. This secondary outcome will use an adjusted proportional odds model to derive the common odds of improvement ("shift") along the mRS scale. Adjustment will include all of the variables in the stratification and minimization algorithm (age, sex, baseline NIHSS score, baseline ASPECTS score, occlusion location, intravenous alteplase treatment, declared first endovascular approach used and site). The proportional odds assumption will be tested.

The choice of the shift analysis is based upon empirical evidence that the mRS scale, when categories 5 and 6 are combined, is a true interval scale meaning that any one step increment in the scale is the same; thus, it is unlikely that the proportional odds assumption will be violated and clinical step changes across the scale are meaningful.[42] The proportional odds approach to analysis of the mRS scale was applied in the ESCAPE trial.[3,43] It allows us to include elderly subject (who have a poorer prognosis irrespective of treatment) because a shift from severe to moderate disability will still be recognized as an important outcome.

Figure 2: Projected outcome by mRS category based data from the ESCAPE trial and estimation of NA-1 effect based on the minimal clinically important difference.



Predicted outcomes by mRS category in the control group are from the ESCAPE trial.[3] This shows an average 2-3% improvement in outcomes within each mRS category.

If the distribution of subject follows that shown in Figure 2 (i.e. optimally shows a shift across the full scale with common OR 1.32), simulation (n=5000 iterations) demonstrates that the power will be 97.2% to show a common OR of 1.32 with 1076 evaluable subject.

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

The fixed sequential order for testing in the ITT trial population is:

1) Primary efficacy outcome
2) Key secondary outcome analysis (shift analysis using proportional odds model);
3) Secondary efficacy endpoints, as specified in the order presented below in Section 11.5.

11.5 Analysis of Secondary Efficacy Outcomes

The key secondary outcomes will be tested in the following order:

1. Shift analysis of 90 day mRS under proportional odds model across the mRS scale, as described in Section 11.4 above.
2. The NIHSS 0-2 vs. 3 or greater at 90 days.
3. The BI at 95-100 vs. 0-90.
4. The mortality rate at 90 days.
5. The proportion of subjects with mRS score of 0-1 at Day 90.

11.5.1 Modified Rankin Scale – Shift Analysis

The mRS shift analysis will be conducted as described in Section 11.4 above.

11.5.2 National Institutes of Health Stroke Scale

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

11.5.3 Barthel Index

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

11.5.4 Mortality Rate

Mortality rates, as defined as the number of deaths observed divided by the number of subject observed over the 90 day study period between NA-1 and placebo control subjects, will be analyzed by the same logistic regression model as in the primary efficacy.

11.5.5 Modified Rankin Scale – 0-1 Dichotomy Analysis

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

11.6 Analysis of Tertiary Efficacy Outcomes

11.6.1 EQ-5D-5L

For the EQ-5D-5L, the difference between NA-1 and placebo control subjects in the distribution of the index and VAS scores on these scales at Day 90 will be presented descriptively.

11.6.2 Volume of Stroke

The total volume of new a) DWI and b) FLAIR lesions in NA-1 versus placebo control subjects will be calculated using a linear regression using a cubic root transformation. Total volume will be assessed using a linear regression using a cubic root transformation if needed. An ANCOVA will be fit and summarized.

Specific covariates are listed in Section 11.7.

11.6.3 Cognitive outcomes

These will be explored using the BNT15, SNAP and the MoCA.

11.6.4 mRS at 30 Days

The overall proportion subjects experiencing a favorable functional outcome 30-days post-randomization, defined as 0 to 2 on the mRS.

The tertiary and all other analyses will be considered exploratory. Details of the methods of analysis will be finalized in the SAP prior to the first IDMC meeting.

11.6.5 Pharmacokinetics

Descriptive statistics will be calculated for plasma concentrations and for all PK parameters (NA-1). In addition, dose proportionality and linearity of dose dependent PK parameters will be investigated. Individual and mean plasma concentration versus time curves will be plotted on linear and semi-logarithmic scales. Plasma concentration versus time curves will be labelled appropriately with treatment regimen and batch number.

11.7 Adjustment for Covariates and Subgroup Analyses

In addition to the primary and secondary analyses adjusting for age, sex, baseline NIHSS score, baseline ASPECTS score, occlusion location, intravenous alteplase use, declared first endovascular device and site, exploratory analyses will be conducted to determine the potential roles of common baseline characteristics and assess potential heterogeneity of treatment effect across subgroups. Specific subgroups of interest include the very elderly (age > 80 years), men vs. women, subject with different baseline stroke severity (on NIHSS and measured radiologically on ASPECTS), subject treated with intravenous alteplase (yes/no), declared first endovascular approach, baseline occlusion location (MCA vs. ICA), and subject with long/short time from onset to treatment. Full details will be specified in detail in the SAP.

11.8 Handling of Missing, Unused and Spurious Data

Every effort will be made to keep missing data, particularly the Day 90 outcome assessments, to a minimum. However, some missing data may be inevitable due to, for example, loss to follow-up. This was kept to a minimum of 1.3% in the ESCAPE trial[3]. Deceased subject will score 6 on the mRS and be counted as non-responders. For the primary analysis for regulatory submission, we will assume that subject missing the primary endpoint data will be considered to be non-responders. Sensitivity analyses using various imputation techniques will be specified prospectively in the SAP before the database lock for the interim analysis if more than 5% of subject randomized are missing the primary endpoint.

11.9 Analyses of Safety

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

11.9.1 SAEs

SAEs over the 90-day study period will be summarized by presenting, for each treatment group, the number and percentage of subjects having at least one SAE, having an SAE in each body

system and preferred term, by severity and relatedness to study medication. 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 NA-1 and placebo control groups will be summarized within treatment group.

11.9.2 Mortality

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

11.9.3 AEs

Additional analyses will consider the frequency of AEs 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 10-1. 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).

11.9.4 Vital Signs

Absolute values and changes for vital signs from pre-dose to Day 5 or discharge will be documented. The maximum deviation of BP from Baseline between drug and placebo control groups (systolic and diastolic) to 24 Hours will be analyzed using ANCOVA, including factors for treatment group.

11.9.5 Laboratory and 12-Lead ECG Results

Absolute values for laboratory results and generalized 12-lead ECG results (i.e., normal, sinus bradycardia, etc.) will be documented descriptively.

11.9.6 Prior and Concomitant Medications

Prior and concomitant medications will be listed per patient, with the listings separated within treatment group.

11.10 Independent Data Monitoring Committee

An IDMC will perform periodic safety reviews of the clinical data. The reviews will occur when 100, 300 and 600 subjects have reached their Day 90 final study visit.

An efficacy interim analysis after approximately 600 subjects complete the Day 90 follow-up will be conducted using the alpha spending function method[1] with O'Brien and Fleming[2] type stopping boundary for efficacy and a non-binding conditional power boundary for futility. The trial may be stopped for overwhelming efficacy or for futility at the interim analysis if the test statistic crosses the O-F or conditional power boundary. The IDMC charter will describe, among other

things, the approach to handling overwhelming efficacy, futility, and potential imbalance in key prognostic variables.

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

12 DIRECT ACCESS TO SOURCE DATA/DOCUMENTS

The sponsor or delegate 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 will maintain regular telephone and written communication with all investigators through the coordinating centre. The sponsor (or delegate) 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. E-CRFs 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 e-CRFs, verification of e-CRF 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 investigators will permit trial-related monitoring, audits, REB/IRB review, and regulatory inspections, providing direct access to source data/documents.

13 QUALITY CONTROL AND QUALITY ASSURANCE

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 Health Insurance Portability and Accountability Act (HIPAA) 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 CRO. 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 patients 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 as directed by the CRU. 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 IRB/REB. Finally, any additional special considerations for the auditing of any additional safety issues are made during this final monitoring visit.

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, ICH-GCP, SOPs and regulatory requirements. All medical treatments will be recorded. Any deviation(s) from the protocol will be recorded and presented in the final clinical study report.

13.1 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.

13.2 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/IRB for approval prior to implementation.

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

14 ETHICS

14.1 Research Ethics Board/Institutional Review Board

This study will be conducted in substantial compliance with the principles and requirements of the ICH-GCP, Canadian Food and Drug Regulations, United States Code of Federal Regulations (CFR; 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 REB/IRB. Before initiation of the study, a copy of the REB/IRBs' approval letters will be provided to the sponsor and the membership list of the REB/IRB will be kept on file.

This protocol and the informed consent document and any subsequent modifications will be reviewed and approved by the REB/IRB 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. SAEs will be reported to the REB/IRB according to their requirements.

15 DATA HANDLING AND RECORDKEEPING

15.1 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.

15.2 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/IRB and governmental approval with correspondence, all versions of ethics approved informed consent forms, staff curriculum vitae and authorization forms and other appropriate documents/correspondence, etc.

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, original 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/IRB.

15.3 Source Documents and Background Data

Any investigators shall supply the sponsor, upon request, with any required background data from the study documentation or clinic records. This is particularly important when e-CRFs 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.

15.4 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.

15.5 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/IRB, 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 [e.g. Health Information Act (HIA) in AB] where applicable, must be followed. Additionally, any U.S. clinical sites must follow privacy obligations to study subjects under HIPAA. European or Asian/Australasian sites must conform to local privacy and confidentiality law and custom. On the CRFs and other study documents or image materials submitted to the CRU, 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.

16 PUBLICATION AND PRESENTATION POLICY

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

17 ANCILLARY STUDIES POLICY

Ancillary or sub-studies may be considered by the trial executive committee. Important principles that guide the addition of ancillary studies are:

1) No subject shall be enrolled in a concurrent investigational drug/device trial during the study period
2) Concurrent enrollment of an ESCAPE-NA1 study subject in a site specific observational cohort study is allowable, where the following conditions are met:
    a. The executive committee is notified
    b. The concurrent study does not interfere with any study follow-up procedures or potentially confound the outcome of the ESCAPE-NA1 trial
    c. The site principal/qualified investigator of the concurrent study explicitly acknowledges that the treatment given in the ESCAPE-NA1 trial may confound the outcome of the site-specific concurrent study
    d. The subject may not be included in any publication or report until the ESCAPE-NA1 study has been concluded
3) Ancillary or sub-studies shall be vetted and approved by the trial executive committee.

18 DATA-SHARING PLAN

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

The Executive Committee will make the trial results available as free-access using PubMed and on Clinical Trials.gov (www.clinicaltrials.gov).

19 FINANCIAL CONSIDERATIONS

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

- Admission to hospital
- Baseline laboratory testing, pregnancy test, ECG, baseline NCCT and CTA, baseline CTP
- Endovascular procedure and angiography
- Follow-up limited-sequence MR brain imaging at 24 Hours
- Follow up ECG at 24 Hours
- Follow-up laboratory testing (other than the mandated tests at 24 hours)
- Physician fees
- Treatment processes in the endovascular lab since they are considered standard of care
- Stroke unit care in hospital
- Nursing care
- Rehabilitation and home care if relevant
- Outpatient clinic follow-up at 90 days (routine)

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

20 Appendix 1 – Additional Guidance on Imaging Selection and Endovascular Treatment

Notes on Eligibility for Endovascular Therapy

Eligibility for endovascular treatment has been defined by the recent randomized clinical trials. Guidance on selection of subject using imaging is as follows:

Exclude subject with evidence of a large core of infarction (ASPECTS 0-4), evidence of very poor intracranial collaterals on CTA:

- A large core can be defined as a baseline NCCT reveals a moderate/large core defined as extensive early ischemic changes of ASPECTS 0-4 in the territory of symptomatic intracranial occlusion.
- Poor collaterals can be defined *on a multiphase or dynamic CTA*: no or minimal collaterals (ie. equivalent to a Tan score 0 or 1). Poor collaterals can be similarly defined on *single phase CTA* but with reduced specificity compared to CTA. Multiphase or dynamic CTA is required for the study.

We advise using the ESCAPE[3] imaging criteria for selection of subject into the trial.

Angiographic Process Measures

Short imaging time:

The total imaging time starts from the time the subject reaches the door of imaging suite to when the subject is out of there and ready to move to next step. It includes putting subject on table, setting intravenous line, connecting to pump, etc. In the case of MR it would include the time taken to exclude presence of pacemaker etc. It includes post-processing time. Imaging time should not exceed 15 minutes.

Excellent overall organization to reduce CT-to-groin puncture time:

There are multiple steps to get from imaging to the groin puncture and these may include:

- Image interpretation
- Blood draw
- ECG
- Anesthesia involvement for conscious sedation
- Prepping the angiography suite
- Availability of nurse and technician
- Availability of respiratory technician and anesthetist if appropriate
- Ability to move the subject from the CT suite rapidly to the interventional suite

Groin puncture-to-recanalization time:

From the time the subject is on angiography suite table:

- Pre-plan your procedure, catheters required by reviewing the CTA of the arch
- Puncture the groin while the subject is getting draped. Do not wait.

- Have a stroke kit ready.
- Have a pre-decided division of labour: who is going to do what?
- Simple things like shaving the groin: is it needed?
- Foley catheter. Draining the bladder improves subject comfort and help reduce BP
- Assess need for anesthesia; overall general anesthesia should be avoided. Involvement of an anesthetist for conscious sedation is ideal.
- Training on use of other drugs to hold subject still: consult with local anesthetist.
- 8F sheath.
- Go straight to vessel of interest.
- Choice of catheter dependent on CTA.
- Whether common carotid artery run needed on not dependent on CTA.
- Try to use standardized microcatheter and wire.
- Plan an entire kit of stuff that is needed included things like a 60 cc syringe.

TICI2c or 3 flow:

Aim for complete recanalization [Thrombolysis in Myocardial Infarction (TIMI) 3] and reperfusion (TICI 3). A few small distal emboli are an acceptable outcome and are generally tolerated well by collateral flow.

Control BP:

While the artery remains occluded, systolic blood pressure (SBP) 150 or greater is probably useful in promoting and keeping collateral flow adequate. Indirect evidence supports this assertion. Once reperfusion has been achieved, BP often falls naturally. Controlling BP once reperfusion has been achieved aiming for a normal BP for that individual is sensible. Labetalol or an intravenous β-blocker such as metoprolol in low doses is preferred.

Keep complication rate low:

Be prepared to stop and back out. Do not take unnecessary risks. Remember that you do not necessarily need a "perfect" angiographic outcome to have a good clinical outcome.

Medical Management:

Designate one person to observe and manage the subject's vital signs. Follow the oxygen saturation (SaO_2), pulse and BP. Manage accordingly. In an ideal circumstance, an anesthetist helps manage this aspect of care.

In the absence of an anesthetist, the standard of care is that the attending neurologist or stroke fellow will have a lead apron on and be in the neuro-angiosuite managing the medical and neurological condition of the subject.

BP management is described above. There are no clear guidelines that are based upon level 1 evidence.

Conscious Sedation Management:

We strongly recommend that all sites avoid general anesthesia. The vast majority of endovascular stroke treatment can be completed under conscious sedation. Several case series support the

concept that general anesthesia is associated with poorer outcomes. A possible explanation is prolonged BP lowering resulting in more rapid progression to infarction.

A simple conscious sedation paradigm for adult subject is as follows:

- 2.5 mg midazolam IV
- 25-50 µg fentanyl IV

These drugs should be given together since this avoids the occasional paradoxical agitation seen with intravenous benzodiazepines alone. They can be given q10-15min. Follow the RR and SaO2 to avoid trouble with respiratory suppression. Managing conscious sedation can be done by the neurologist or stroke fellow in the room.

It is often additionally helpful to pre-treat subject with anti-nausea agents:

- 25-50 mg dimenhydrinate (gravol) IV [available in Canada, not the US]

This drug is very mildly sedating and may help avoid an episode of aspiration.

The only subject who typically will require intubation include those with paradoxical agitation associated with a Wernicke's aphasia, or those who develop respiratory compromise due to aspiration or other event arising after randomization.

Carotid artery stenting:

It is recommended as a guideline that carotid artery stenting is should not be done as part of the treatment in the study. Tandem occlusion of the extracranial carotid at the bifurcation associated with an M1-MCA occlusion is common. Patients with tandem occlusions are eligible for enrolment. The target lesion initially is the M1-MCA occlusion. Advance a catheter past the ICA lesion and deal with the M1-MCA first. Very often, in this process, the proximal ICA is open at the end of treating the M1-MCA because of the mechanical manipulation of the occlusive lesion at the carotid. What was initially a 99% stenosis is reduced to 60 or 70%.

At this point stop. Treat the subject with ASA per rectum (since they cannot swallow) and bring the subject back the following day to decide on whether subsequent procedures are necessary. It is simply not necessarily true that forward flow in the carotid is required to keep the M1-MCA open.

The major potential difficulty with leaving a carotid stent in place is the need for antiplatelet therapy, which will increase the chance of major symptomatic bleeding.

If, despite the above, the treating physician deems that a stent is required – either intracranially or at the carotid bifurcation because this action is in the patients' best interest, we recommend using ASA and clopidogrel rather intravenous abciximab or eptifibatide. Give ASA per rectum and as soon as possible give 600 mg clopidogrel per NG.

Use of IA medications:

Use of intra-arterial medication such as alteplase, abciximab, epitifibatide, tirofiban is not advised although nothing in this protocol forbids the use of any treatment that the treating physician deems to be in the subject's best interest. Any such medications will be noted in the CRF. Note that alteplase is not indicated for direct IA catheter delivery.

Number of Devices / GP2b3a Inhibitors / When to stop:

Some lesions are too difficult to treat and adequate recanalization may not be achieved. The more devices that are used and the most antithrombotic agents that are used, the greater the risk of major AEs. Use of intravenous GP2b3a inhibitors in the setting of AIS, is associated with a very high risk (up to 40%) of hemorrhage. Please do not use them. Please make every effort to achieve recanalization within 90 minutes of imaging. If this is clearly not going to be achievable, the interventionalist and team must make a judgment as to whether to continue. Spending more than 90 minutes working on an intracranial lesion is a recipe for major AEs.

Chasing distal M2, M3 or A2, A3 occlusions:

Once the arterial occlusive lesion is open, there will remain visible distal occlusion approximately 10-20% of the time. Do not chase these. Do not give regional alteplase infusion. A perfect angiographic outcome is not necessary for a good clinical outcome and there is a greater risk of doing harm.

21 Appendix 2: Derivation of ASPECTS on NCCT and Collaterals Scoring on CTA

Minimal Acceptable Quality for CT scan

There is potential for variation across sites as to the choice of imaging modalities that treating physicians use for deciding appropriate subject for intervention. Overall, CT is the mainstay for this trial and all patients must be selected on the basis of CT and multiphase or dynamic CTA, to standardize imaging patient selection criteria.

- NCCT
- CTA from arch to head

NCCT quality

- Minimum power: 120-140 kV, 170-200 mA
- Two second scanning time
- 5 mm section thickness
- Appropriate algorithm reducing bone artefacts and high SNR for gray-white differentiation
- Contiguous axial sections from skull base to vertex parallel to the inferior orbitomeatal line (IOML)
- A good quality stroke protocol scan is defined by the following two criteria on the unaffected side: Lateral margin of the lentiform nucleus well discriminated in the absence of previous infarction.
- Insular ribbon is well defined in the absence of previous infarction.
- CTA should use minimum contrast media –75cc is the recommended amount
- Helical acquisition
- Reformats should include: thin (3mm) axial, sagittal, coronal maximum intensity projection (MIPS), thick (25 mm) axial and sagittal MIPS

NCCT

NCCT shall be scored using ASPECTS, a 10-point score derived by examining each of 10 regions on the middle cerebral artery territory. Ischemic change present is scored as 0; ischemic change absent is score as 1. Adding up the score gives a maximum of 10 (favourable scan) and minimum of 0 (unfavourable scan).

The score is highly reliable when trichotomized into 0-4 (severe ischemic change, large core), 5-7 (moderate ischemic change) and 8-10 (minimal ischemic change, small core). ASPECTS may be less reliable early in stroke (i.e. within 90 minutes of onset); however, at later time windows it should be easy to recognize large areas of irreversible damage. Having a good quality scan and optimization of scanner is key to successful interpretation. Further information is available at: www.aspectsinstroke.com.

CTA

The importance of contrast bolus timing should be emphasized. Using single-phase CTA, it is important to have a sufficiently venous weighted study to be able to visualize the collateral

circulation adequately. The CTA must include the aortic arch to allow the interventionalist a view for planning.

Multi-phase (3 or 4 phase) CTA will be used to show the evolution of the blood flow. This technique is particularly useful in demonstrating regional blood flow hang-up and implicated distal circulation occlusions. The timing of mCTA imaging depends upon the individual CT scanner and table speed. By vendor, there is approximately 5-8 seconds between phases. See: www.aspectsinstroke.com.

Scoring of Collateral Status

The CTA will be assessed for collateral status using a standardized technique similar to the ESCAPE trial. Collaterals are measured on multi-phase CTA by comparing backfilling pial vessels beyond the blocked artery to similar vessels in the opposite, unaffected hemisphere (Figure A1). This is done for all phases of the CTA, which depending upon the machine type will vary from 5-8 seconds apart in time. Vascular enhancement distal to occlusion is scored in anterior and posterior MCA territories as visualized on all CTA slices, and typically reviewed on axial thick maximal intensity projections (MIPs). By examining all 3 phases, the degree of filling of the pial vessels in time can be assessed. Delayed filling and delayed washout ipsilateral to the occluded artery is expected. The aim in the ESCAPE-NA1 trial is to exclude patients with absent or near absent pial collateral filling on all phases of the CTA. Phase 1 (early) absence or minimal filling with later (phase 2 and 3) robust filling represents strong collaterals and an ideal treatment candidate.

The following scoring system provides a guide. Patients with a score of 0 or 1 will be excluded for this study.

Category	Score	Description
Good	5	Compared to asymptomatic contralateral hemisphere, there is no delay and normal or increased prominence of peripheral vessels/ normal extent within the occluded arteries territory within the symptomatic hemisphere.
	4	Compared to asymptomatic contralateral hemisphere there is a delay of one phase in filling in of peripheral vessels but prominence and extent is the same.
Intermediate	3	Compared to asymptomatic contralateral hemisphere there is a delay of two phases in filling in of peripheral vessels but prominence and extent is the same or there is a one phase delay and decreased prominence (thinner vessels) / reduced number of vessels in some part of the territory occluded.
	2	Compared to asymptomatic contralateral hemisphere there is a delay of two phases in filling in of peripheral vessels and decreased prominence and extent or a one-phase delay and some regions with no vessels in some part of the territory occluded.
Poor	1	Compared to asymptomatic contralateral hemisphere there are just a few vessels visible in any phase within the occluded vascular territory.
	0	Compared to asymptomatic contralateral hemisphere there are no vessels visible in any phase within the occluded vascular territory.

*On a *single phase, multiphase or dynamic CTA:* no or minimal collaterals in a region greater than 50% of the MCA territory when compared to pial filling on the contralateral side can also be considered as poor collaterals (Score = 1).

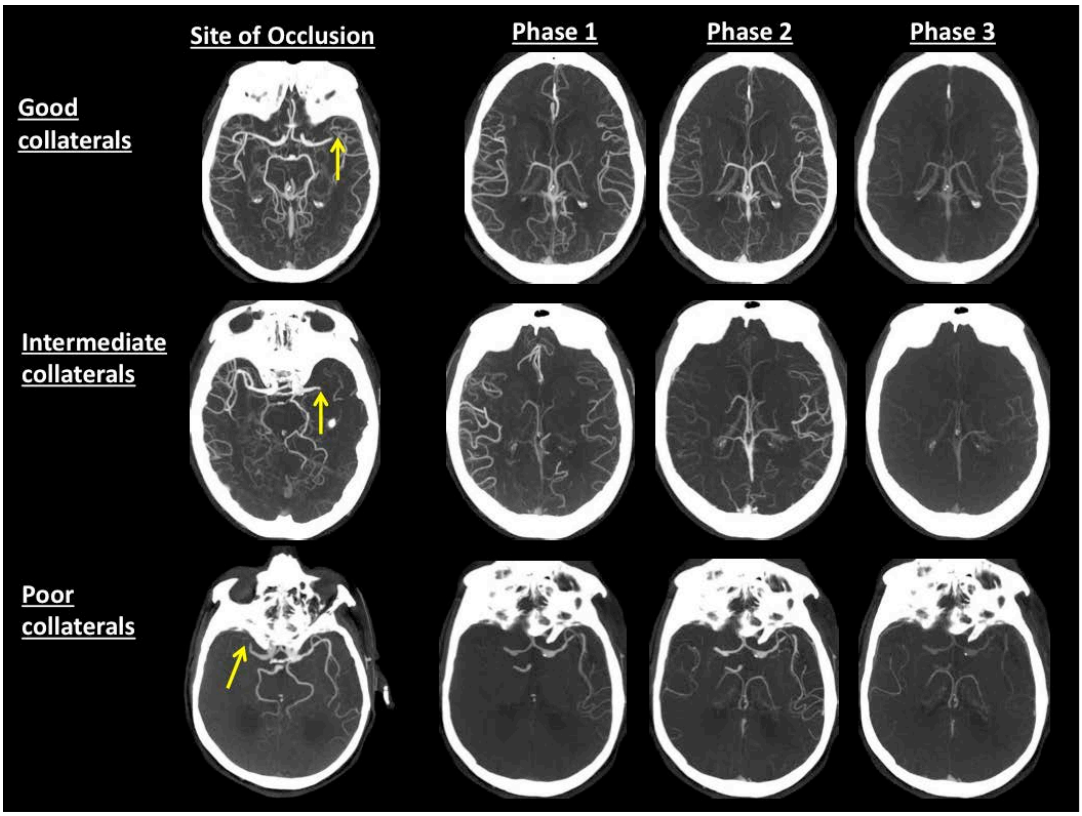


Figure A1. Upper panel shows a patient with a left M1 MCA occlusion (arrow) and good collaterals (backfilling arteries) on multi-phase CTA. Middle Panel shows a patient with a left M1 MCA occlusion (arrow) and intermediate collaterals. Lower panel shows a patient with a right M1 MCA occlusion (arrow) and poor collaterals (minimal backfilling arteries) on multi-phase CTA.

A teaching slide set and module will be available for use within the trial.

22 Appendix 3 – Randomization Details.

The initial randomization of the first 40 subject will be completed using a simple random number generator yielding an unblocked randomization with an equal probability for each treatment arm. Thereafter, randomization will be stratified by intravenous alteplase use and declared endovascular approach combined with a randomized minimization method from Zhao et al, called the minimal sufficient balance method[23]. This approach will ensure that the subjects entered into the trial will be matched between control and active treatment arms on the key variables of age, sex, baseline NIHSS score, baseline ASPECTS score, occlusion location (MCA or ICA), and site. This method will ensure that during the trial and by the end of the trial, the subjects are well matched for important prognostic variables and therefore the chance that any treatment effect is confounded by a specific variable is severely attenuated, because the groups are balanced on known important prognostic variables. Minimization will also provide some assurance that adequate balance will be maintained even at the time of interim analysis.

A randomization website, using user and site level secure access, will assign treatment in real time. Five key clinical variables (age, sex, baseline NIHSS score, baseline ASPECTS score, occlusion location (MCA or ICA)), and one site variable, defined by the user log in credentials, will be used in the minimization procedure. Age will be calculated against the central server date and time, which will be synchronized weekly to the Denver atomic clock. In this method, balance will be checked on the six variables using a simple t-test for continuous variables and a $\chi 2$ test for binary variables. If the p-value for the test is <0.3, then imbalance is considered present and a vote is given to that variable to randomize to Group A or Group B to minimize imbalance. If the summation of six votes favours, for example, Group A allocation, then a biased coin randomization probability of 0.65 is used and the probability of allocation to Group A becomes 0.65. If the votes are equal, then the probability remains 0.5. This methodology was successfully used in the ESCAPE trial[3]. A permutation test for covariate balance was undertaken at the end of the trial and showed no evidence of biased treatment allocation. This same approach will be repeated in the ESCAPE-NA1 trial to demonstrate that the minimization algorithm was effective. Treatment assignment will always be fully concealed since it will be assigned in real-time based upon random number generation.

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24 Investigator's Agreement

I have read the attached protocol: **A Multicentre, Randomized, Double-blinded, Placebo-controlled, Parallel Group, Single-dose Design to Determine the Efficacy and Safety of Intravenous NA-1 in Subjects with Acute Ischemic Stroke Undergoing Endovascular Thrombectomy (ESCAPE-NA1 Trial),** Version 9.0 dated 19 February 2019 and agree to abide by all provisions set forth therein.

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

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

Name Site Principal Investigator	Signature
Name of Clinical Site	Date