Clinical Study Protocol

PROTOCOL NUMBER:	DM199-2017-001
PROTOCOL TITLE:	A Randomized, Double-blind, Placebo-controlled Phase II Multi-Center Evaluation to Assess the Safety and Tolerability of DM199 Administered Intravenously and Subcutaneously in Subjects with Acute Ischemic Stroke (ReMEDy Trial)
STUDY PHASE:	II
INVESTIGATIONAL PRODUCT:	DM199
SPONSOR:	DiaMedica Australia Pty Ltd 58 Gipps Street Collingwood, VIC 3066 Australia
NATIONAL PRINCIPAL INVESTIGATOR:	Bruce Campbell, MD Royal Melbourne Hospital 300 Grattan Street Parkville, VIC 3050 Australia
RESPONSIBLE CRO:	Novotech Level 3, 235 Pyrmont Street Pyrmont, NSW 2009 Australia
PROTOCOL VERSION & DATE:	Version 1.1, 20 September 2017

PROTOCOL AGREEMENT FORM

A Randomized, Double-blind, Placebo-controlled Phase II Multi-Center Evaluation to Assess the Safety and Tolerability of DM199 Administered Intravenously and Subcutaneously in Subjects with Acute Ischemic Stroke (ReMEDy Trial)

Version 1.1

20 September 2017

I confirm that I have read the above referenced protocol and understand its contents. The information it contains is consistent with the current risk-benefit evaluation of the investigational product.

I agree to conduct the study according to this protocol and to comply with its requirements, subject to scientific, ethical, and safety considerations. I will obtain the required regulatory and Institutional Review Board/Ethics Committee (IRB/EC) approvals prior to initiating the study and will abide by any additional requirements imposed by the IRB/EC. I will provide copies of this clinical study protocol and all pertinent information to the study personnel under my supervision and will discuss this material with them and ensure they are fully informed regarding the investigational product and the conduct of the study. I agree to conduct the study in compliance with ICH E6 Guidance for Good Clinical Practice (GCP), applicable privacy laws, Declaration of Helsinki, US Code of Federal Regulations, Title 21, Parts 50, 54, 56, and 312, and according to applicable local requirements.

I understand that I must keep confidential the information contained in study documents that I will be provided with over the course of the study.

I understand that, should the decision be made by the Sponsor to prematurely terminate or suspend the study at any time for any reason, such a decision will be communicated to me in writing. Should I decide to withdraw from participating in the study, I will immediately communicate such decision in writing to the Sponsor.

_____

Clinical Site Name

_____	_____	
Site Principal Investigator (Print Name)	Site Principal Investigator (Signature)	Date

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LIST OF ABBREVIATIONS

Abbreviation	Definition
ACE	Angiotensin converting enzyme
ACEi	Angiotensin converting enzyme inhibitors
ADA	Anti-drug antibodies
ADL	Activities of daily living
AE	Adverse event
ALT	Alanine aminotransferase
ALP	Alkaline Phosphate
aPTT	Activated partial thromboplastin time
ARB	Angiotensin Receptor Blockers
AST	Aspartate aminotransferase
BMI	Body mass index
CBER	Center for Biologics Evaluation and Research
CRP	C-reactive protein
CTCAE	Common Terminology Criteria for Adverse Events
DMP	Data management plan
EC	Ethics Committee
ECG	Electrocardiogram
eCRF	Electronic case report form
EDC	Electronic data capture
ER	Emergency room
GCP	Good Clinical Practice
HbA1c	Glycosylated hemoglobin
HBsAg	Hepatitis B surface antigen
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
HR	Heart rate
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
INR	International normalized ratio
IV	Intravenous
IWRS	Interactive Web Response System
KLK1	Kallikrein-1
LAR	Legally authorized representative
LDH	Lactate dehydrogenase

Abbreviation	Definition
LLOQ	Lower limit of quantitation
MAD	Multiple ascending dose
MAP	Mean arterial blood pressure
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
MedDRA	Medical dictionary for regulatory activities
MMP-9	Matrix metalloproteinase - 9
NIH	National Institutes of Health
NIHSS	National Institutes of Health Stroke Scale/Score
NOAEL	No observable adverse effect level
PBS	Phosphate buffered saline
PD	Pharmacodynamic(s)
PK	Pharmacokinetic(s)
PNAU	P-nitroaniline unit
PT	Prothrombin time
rhKLK1	Recombinant human kallikrein-1
SAD	Single ascending dose
SAE	Serious adverse event
SAP	Statistical analysis plan
SC	Subcutaneous
SOP	Standard operating procedure
TEAE	Treatment-emergent adverse event
tPA	Tissue plasminogen activator
VEGF	Vascular epithelial growth factor

PROTOCOL SYNOPSIS

PROTOCOL NUMBER	DM199-2017-001
PROTOCOL TITLE	A Randomized, Double-blind, Placebo-controlled Phase II Multi-Center Investigation to Assess the Safety and Tolerability of DM199 Administered Intravenously and Subcutaneously in Subjects with Acute Ischemic Stroke
STUDY PHASE	II
INVESTIGATIONAL PRODUCT (IP)	DM199
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NATIONAL PRINCIPAL INVESTIGATOR	Bruce Campbell, MD Royal Melbourne Hospital 300 Grattan Street Parkville, VIC 3050, Australia
RESPONSIBLE CRO	Novotech Level 3, 235 Pyrmont Street Pyrmont, NSW 2009, Australia
CENTRAL LABORATORY	Sonic Clinical Trials 14 Giffnock Avenue Macquarie Park, NSW 2113, Australia
STUDY RATIONALE	DM199 is a recombinant human version of the KLK1 protein. In China, a urinary-derived form of this protein (Kailikang®) is approved as a treatment for acute ischemic stroke based on at least one large randomized, double-blind, placebo-controlled trial. The primary mechanism of KLK1 treatment appears to be an improvement of blood flow in capillaries and formation of new capillaries over the longer term. DM199 has been shown to be safe and well-tolerated in multiple Phase I and Phase IIA clinical trials. Moreover, these trials have demonstrated that DM199 can be dosed to achieve plasma exposures similar to those of Kailikang®. DiaMedica Therapeutics Inc. is conducting this Phase II study in patients with acute ischemic stroke to establish safety and tolerability in this patient population. This study will also explore the effect of treatment on standard efficacy endpoints and biomarkers measured at various time points after the stroke. IV and subcutaneous routes of administration will be tested.
STUDY DESIGN	This is a randomized, double-blind, placebo-controlled Phase II, multi-center study of DM199. Subjects presenting with acute ischemic stroke will be randomized 1:1 to placebo or DM199

	administered by a single intravenous (IV) dose followed by subsequent subcutaneous (SC) doses.
OBJECTIVE	The objective of this study is to evaluate the safety and tolerability of DM199 in treating subjects presenting with acute ischemic stroke.
PRIMARY ENDPOINTS	1. Safety as assessed by: • Incidence, severity and causality of adverse events (AEs) and serious adverse events (SAEs) • Physical examination changes from baseline (days 4, 22, 56, and 90) • Vital sign changes from baseline (resting heart rate, systolic/diastolic blood pressure, respiratory rate, and temperature) (days 1, 4, 7, 10, 13, 16, 19, 22, 56, and 90) • Hematology and chemistry parameter changes from baseline (days 4, 22, 56, and 90) • 12 lead ECG changes from baseline (days 1, 4, and 22) 2. Tolerability as assessed by incidence and severity of injection site adverse events
SECONDARY ENDPOINTS	1. Efficacy as assessed by: • NIH Stroke Scale (days 22 and 90) • Barthel Index (days 22 and 90) • Modified Rankin Scale (days 22 and 90) 2. Plasma concentration of DM199 (days 2, 22, and 90) 3. Pharmacodynamics as assessed by: • Plasma C-reactive protein (CRP) (days 2, 22, and 90) • Plasma MMP-9 (days 2, 22, and 90) • Plasma VEGF (days 2, 22, and 90) • Plasma DM199 ADA (days 2, 22, and 90)
EXPLORATORY ENDPOINTS	1. Urinary KLK1 concentration (days 4, 13, 22, and 90) 2. Pharmacodynamics as assessed by: • Plasma tPA (days 2, 22, and 90) • Plasma Nitric Oxide as NOx (days 2, 22, and 90) • Plasma Prostaglandins (E2) (days 2, 22, 90)

SAMPLE SIZE & SAMPLE SIZE DETERMINATION	No formal sample size estimation was performed. Up to 66 subjects will be enrolled to achieve approximately 60 completed subjects, based on an estimated drop-out rate of 10%. Subjects will be randomized 1:1 to receive DM199 or placebo, resulting in 33 enrolled subjects per treatment group. A sample size of 30 completed subjects in each treatment group is considered adequate to evaluate the safety and tolerability of DM199.
INCLUSION CRITERIA	1. Subject is ≥ 18 years of age. 2. Subject has been diagnosed with acute ischemic stroke with onset ≤ 24 hours from enrollment. 3. Subject has NIH stroke score (NIHSS) ≥ 6 and ≤ 25. *NOTE: For subjects who have received treatment with either tPA (or similar) or any type of mechanical intervention the assessment of the NIHSS must occur at least 1 hour after such treatment for purposes of evaluating eligibility for this study.* 4. Subject or legally authorized representative is willing and able to sign written informed consent.
EXCLUSION CRITERIA	1. Subject is currently prescribed angiotensin-converting-enzyme inhibitors (ACEi) and is unable or unwilling to convert to another antihypertensive pharmacological treatment for the duration of the study. 2. Subject has a history of significant allergic diathesis such as urticaria, angioedema or anaphylaxis. 3. Subjects with current malignancy or active malignancy ≤ 5 years prior to enrollment except basal cell or squamous cell carcinoma of the skin or in situ cervical cancer that has undergone potentially curative therapy and at least six months have elapsed since the procedure. 4. Subject has a history of clinically significant acute bacterial, viral, or fungal systemic infections in the last four weeks prior to enrollment. 5. Subject has clinical or laboratory evidence of an active infection at the time of enrollment. 6. Subject has known alpha 1-antitrypsin deficiency (α1-antitrypsin deficiency). 7. Subject has a known diagnosis of human immunodeficiency virus (HIV), hepatitis B surface antigen (HBsAg), or anti-hepatitis C virus (Anti-HCV) at screening.

	8. Subject is pregnant or nursing. 9. Subject is male or female of childbearing potential, is participating in heterosexual sexual activity that could lead to pregnancy, and is unable or unwilling to practice medically effective contraception during the study. *NOTE: Subjects should agree to use two reliable methods of contraception (e.g., double-barrier condom plus diaphragm, condom or diaphragm plus a stable dose of hormonal contraception) throughout the study period and until three months after receiving study treatment.* *NOTE: A negative urine pregnancy test will be documented during screening.* 10. Subject is participating in any other drug study ≤ 4 weeks or 5 half-lives of the investigational product, whichever is longer. 11. Subject does not have sufficient venous access for infusion of study treatment or blood sampling. 12. In the opinion of the Investigator, subject is unlikely to be followed for the duration of the study. 13. Subject is unable or unwilling to comply with protocol requirements, including assessments, tests, and follow-up visits. 14. Subject has any other medical condition which in the opinion of the Investigator will make participation medically unsafe or interfere with the study results.

1 INTRODUCTION

1.1 BACKGROUND

Acute ischemic stroke is a debilitating disease that is a major medical and economic concern among nations whose demographics include an aging general population. At present, the only pharmacological treatment for stroke is tissue plasminogen activator (tPA) which must be given within 4.5 hours of the ischemic event, where it has been shown to significantly improve function vs placebo in many patients (Cheng & Kim, 2015). Unfortunately, there are currently no medical therapies available to provide further functional improvement beyond tPA, for use after the 4.5-hour window when tPA cannot be administered.

Kallikrein (specifically, tissue kallikrein known as KLK1) is an endogenous enzyme that produces kinins and is the most potent natural vasodilative mechanism known acting through the Bradykinin 1 and 2 receptors. The kinins, including Bradykinin (BK), are rapidly degraded in the blood by ubiquitous peptidases, most notably angiotensin converting enzyme (ACE). Indeed, many of the benefits ascribed to ACE inhibitors (ACEi) appear to involve increases in BK levels.

KLK1 has been shown to protect against ischemic brain injury through multiple signalling pathways including anti-inflammation, anti-apoptotic effect, promoting angiogenesis and neurogenesis, and improved cerebral blood flow through vasodilation. In addition to ischemic brain injury, research on hind limb ischemia, cardiac infarction, and renal ischemia further indicates that kallikrein may be a novel angiogenic protein. For stroke patients, improving cerebrovascular perfusion, dilation of the arteriole, decreasing inflammation, and formation of new vessels in the penumbra region remains a good option to help rescue injured tissue and alleviate neurologic deficits. KLK1 appears to endow these properties.

DiaMedica Therapeutics Inc. (DiaMedica) has developed a recombinant form of KLK1 (rKLK1, DM199) and has previously completed single ascending dose (SAD) and multiple ascending dose (MAD) trials of the subcutaneous dose form. In addition, a small pilot trial in patients with mild type 2 diabetes was conducted over 28 days. To date, DM199 has been administered to 104 healthy volunteers and patients. In all the trials, DM199 proved to be safe and well tolerated. At very high doses (0.3-0.5 mg/kg), hypotension was observed which was expected and consistent with the mechanism of action. No anti-drug antibodies were observed at 28 days.

1.2 NONCLINICAL SUMMARY

The pharmacokinetics (PK) of DM199 was characterized in male and female cynomolgus monkeys (2 males and 2 females) following subcutaneous (SC) injection at 1.80 mg/kg/day for 14 days. The C_{max} of DM199 was reached at 2 hours post-dose for the females and generally by 4 hours for the males on Days 2 and 14. A decrease was observed in the mean exposure parameters with accumulation ratios ranging from 0.198 to 0.380-fold. The terminal elimination phase could not be characterized for any of the profiles. No consistent gender differences in systemic exposure were observed. The PK of DM199 in plasma was also characterized in male Sprague-

Dawley rats when delivered by a single dose intravenously (IV) and SC at dose levels of 0.01049 and 0.1049 mg/kg. For 0.1049 mg/kg by SC administration, peak DM199 mean plasma concentration was observed at 12 hours followed by a shallow mono-exponential decline where the terminal elimination phase was not reached. For IV administration, t_{max} was observed at 15 minutes for 0.01049 mg/kg and 5 minutes for 0.1049 mg/kg. Limited exposure did not allow for characterization of the terminal phase at 0.01049 mg/kg by IV administration. For 0.1049 mg/kg by IV administration, the peak DM199 plasma concentration was followed by a mono-exponential decline and $t_{1/2}$ was estimated to be 6.44 hours. Absolute bioavailability using C_{max} (SC/IV) was 53% for the 0.1049 mg/kg dose level.

There were no observed toxicity effects of DM199 in Sprague-Dawley rats when given by SC injection once during an escalating dose at a dose level up to 3.69 mg/kg. In exploratory non-GLP single dose escalation study in cynomolgus monkeys, DM199-related findings were limited to a decrease in mean arterial blood pressure (MAP) at the high dose (3.6 mg/kg) compared to the control dose. Due to the magnitude of the drop in MAP, the 3.6 mg/kg dose was considered to be the observed adverse effect dose.

In repeated dosing studies, administration of DM199 by SC injection at dose levels of 1.44, 2.88 and 3.6 mg/kg/day for 28 days was well tolerated in rats. There were no DM199 associated findings for clinical observations, body weights, food consumption, ophthalmic observations, hematology, clinical chemistry, coagulation, urinalysis, gross pathology observations, or organ weights and ratios. Administration of DM199 by SC injection for 1 month was well tolerated in cynomolgus monkeys at all dose levels. A single high dose female animal was euthanized for humane reasons on Day 8 for telemetry implant-related complications that were not due to test article administration. DM199-related findings were limited to a non-dose-related microscopic finding at the injection site at all dose levels and an increase in liver-specific enzyme serum levels at 1.44 mg/kg/day. Based on these results, the no observable adverse effect level (NOAEL) was considered to be 1.44 mg/kg/day.

Treatment with DM199 in rodent models of type 2 diabetes mellitus restored insulin sensitivity and improved insulin secretion. Treatment of non-obese diabetic mice attenuated the development of autoimmune reaction that results in type 1 diabetes mellitus. At 7 weeks of treatment, the non-obese diabetic mice developed a 6-fold increase in circulating C-peptide without a detectable increase in insulin, episodes of hypoglycemia or increase in beta cell mass.

1.3 CLINICAL SUMMARY

The same formulation of DM199 has been tested in Phase I and Phase II clinical studies. A single ascending dose study was conducted to assess the safety and pharmacokinetics of DM199 in healthy volunteers. This was a double-blind placebo-controlled trial including a total of 18 volunteers between the ages of 20 and 64. DM199 was tested at doses of 1.5, 5, 15, 30 and 50 µg/kg administered subcutaneously. These doses were generally safe and well-tolerated. Drug-

related side effects were observed after the 50 µg/kg dose and were associated with postural hypotension.

A multiple ascending dose study was conducted in healthy volunteers to assess the pharmacokinetics, safety and tolerability of DM199 after chronic dosing once every 3 days for 16 days (6 doses total). The study included 18 volunteers between the ages of 21 and 64, who received ascending doses of 3, 15 and 25 µg/kg over the course of the study by subcutaneous injection. DM199 was safe and well-tolerated at all doses.

DM199 was tested in a Phase II study involving 37 patients with Type 2 Diabetes. Patients were treated with either placebo, 15 and 30 µg/kg DM199 administered subcutaneously for 28 days. The primary endpoint of blood glucose concentration was not different between groups treated with placebo or either dose of DM199. There were 15 adverse events that were considered related to study treatment. All were deemed mild to moderate (Grade 1-2) in severity. These included two cases of hypoglycemia, one observation of postural hypotension, two observations of a mild headache and eight reports of injection site irritation. There was a single severe adverse event (seizure) that was considered to be not related to study treatment.

An open label, Phase IB, single center, ascending dose study was completed to assess the safety and tolerability of DM199. The purpose of the study was to characterize the pharmacokinetics and pharmacodynamics after IV and SC dosing in healthy subjects. A total of 36 subjects were enrolled. The initial component of this study was a single ascending study focusing on a 30-minute infusion of DM199 at doses of 0.25, 0.5, 0.75 and 1 µg/kg. All doses were considered to be safe and well-tolerated. The pharmacokinetic profile of 0.75 µg/kg most closely matched that of urinary KLK1 (Kailikang®), a product that is currently marketed in the People's Republic of China for treating acute ischemic stroke. The second component of the study directly compared an IV infusion of 0.75 µg/kg DM199 to a 3 µg/kg subcutaneous injection. Both approaches generated similar peak plasma concentrations, but the DM199 plasma exposure was substantially longer after subcutaneous injection compared to IV administration. These doses were considered safe and well-tolerated with very few reported adverse events. The dosing strategy for this study is founded on these pharmacokinetic results and is designed to rapidly generate presumed therapeutic concentrations of DM199 and maintain that concentration for the subsequent 22 days of treatment.

1.4 RATIONALE

DM199 is being developed as a new biological treatment for acute ischemic stroke. The purpose of this Phase II study is to assess safety and tolerability of DM199 in patients with acute ischemic stroke.

2 STUDY DESIGN

This is a randomized, double-blind, placebo-controlled Phase II, multi-center study of DM199. Subjects presenting with acute ischemic stroke will be randomized 1:1 to placebo or DM199

administered by a single intravenous (IV) dose followed by subsequent subcutaneous (SC) doses. Figure 1 depicts the overall study design.

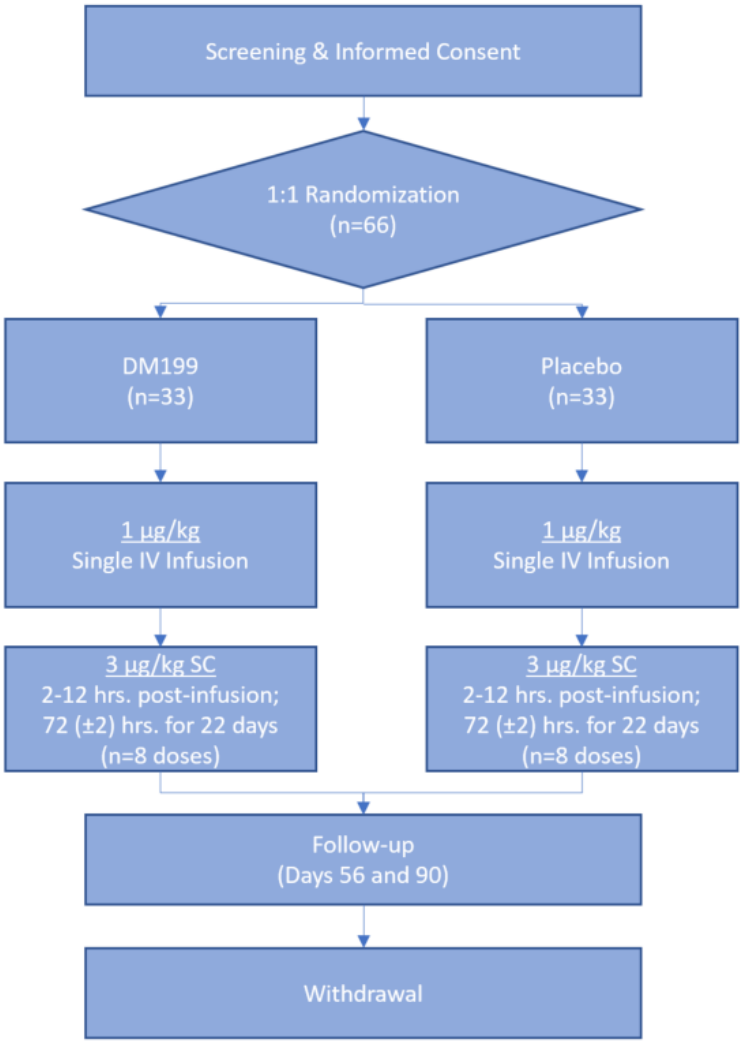

Figure 1. Schematic Study Design

2.1 OBJECTIVE

The objective of this study is to evaluate the safety and tolerability of DM199 in treating subjects presenting with acute ischemic stroke.

2.2 ENDPOINTS

2.2.1 Primary Endpoints

The primary endpoint of this study is to evaluate the safety and tolerability of DM199. Endpoints will be assessed as follows:

1. Safety:
    - Incidence, severity and causality of adverse events (AE) and serious adverse events (SAEs)
    - Physical examination changes from baseline (days 4, 22, 56, and 90)
    - Vital sign changes from baseline (resting heart rate, systolic/diastolic blood pressure, respiratory rate, and temperature) (days 1, 4, 7, 10, 13, 16, 19, 22, 56, and 90)
    - Hematology and chemistry parameter changes from baseline (days 4, 22, 56, and 90)
    - 12 lead ECG changes from baseline (days 1, 4, and 22)
2. Tolerability as assessed by incidence and severity of injection site adverse events.

2.2.2 Secondary Endpoints

The secondary endpoints of this study are to evaluate:

1. Efficacy as assessed by:
    - NIH Stroke Scale (days 22 and 90)
    - Barthel Index (days 22 and 90)
    - Modified Rankin Scale (days 22and 90)
2. Plasma concentration of DM199 (days 2, 22, and 90)
3. Pharmacodynamics as assessed by:
    - Plasma C-reactive protein (CRP) (days 2, 22, and 90)
    - Plasma MMP-9 (days 2, 22, and 90)
    - Plasma VEGF (days 2, 22, and 90)
    - Plasma DM199 ADA (days 2, 22, and 90)

2.2.3 Exploratory Endpoints

Data will be collected on additional exploratory endpoints in which no hypothesis is being made with respect to the ability of the investigational product. Exploratory endpoints include:

1. Urinary KLK1 concentration (days 4, 13, 22, and 90)
2. Pharmacodynamics as assessed by:
    - Plasma tPA (days 2, 22, and 90)
    - Plasma Nitric Oxide as NOx (days 2, 22, and 90)

- Plasma Prostaglandins (E2) (days 2, 22, and 90)

2.3 NUMBER OF SUBJECTS

Up to 66 subjects will be enrolled to achieve approximately 60 completed subjects, based on an estimated drop-out rate of 10%. Subjects will be randomized 1:1 to receive DM199 or placebo, resulting in 33 enrolled subjects per treatment group. A sample size of 30 completed subjects in each treatment group is considered adequate to evaluate the safety and tolerability of DM199. Subjects may be enrolled at up to twelve sites globally. Should a subject drop out, the drop out subject may be replaced by a subject assigned to the same treatment arm as the drop out subject.

2.4 STUDY DURATION

The duration of each subject's individual participation in the study will be 90 days from the time of consent to completion of all study activities. Subjects will receive 22 days of active treatment and will be followed for an additional 68 days.

It is estimated that approximately twelve months will be needed to recruit the total number of study subjects.

3 STUDY POPULATION

3.1 INCLUSION CRITERIA

A potential subject must meet all inclusion criteria to be enrolled in the study:

1. Subject is ≥ 18 years of age.
2. Subject has been diagnosed with acute ischemic stroke with onset ≤ 24 hours from enrollment.
3. Subject has NIH stroke score (NIHSS) ≥ 6 and ≤ 25.

   NOTE: For subjects who have received treatment with either tPA (or similar) or any type of mechanical intervention the assessment of the NIHSS must occur at least 1 hour after such treatment for purposes of evaluating eligibility for this study.
4. Subject or legally authorized representative is willing and able to sign written informed consent.

3.2 EXCLUSION CRITERIA

Potential subjects will be excluded from the study if any of the conditions apply:

1. Subject is currently prescribed angiotensin-converting-enzyme inhibitors (ACEi) and is unable or unwilling to convert to another antihypertensive pharmacological treatment for the duration of the study.

2. Subject has a history of significant allergic diathesis such as urticaria, angioedema or anaphylaxis.
3. Subjects with current malignancy or active malignancy ≤ 5 years prior to enrollment except basal cell or squamous cell carcinoma of the skin or in situ cervical cancer that has undergone potentially curative therapy and at least six months have elapsed since the procedure.
4. Subject has a history of clinically significant acute bacterial, viral, or fungal systemic infections in the last four weeks prior to enrollment.
5. Subject has clinical or laboratory evidence of an active infection at the time of enrollment.
6. Subject has known alpha 1-antitrypsin deficiency (α1-antitrypsin deficiency).
7. Subject has a known diagnosis of human immunodeficiency virus (HIV), hepatitis B surface antigen (HBsAg), or anti-hepatitis C virus (Anti-HCV) at screening.
8. Subject is pregnant or nursing.
9. Subject is male or female of childbearing potential, is participating in heterosexual sexual activity that could lead to pregnancy, and is unable or unwilling to practice medically effective contraception during the study.

   NOTE: Subjects should agree to use two reliable methods of contraception (e.g., double-barrier condom plus diaphragm, condom or diaphragm plus a stable dose of hormonal contraception) throughout the study period and until three months after receiving study treatment.

   NOTE: A negative urine pregnancy test will be documented during screening.
10. Subject is participating in any other drug study ≤ 4 weeks or 5 half-lives of the investigational product, whichever is longer.
11. Subject does not have sufficient venous access for infusion of study treatment or blood sampling.
12. In the opinion of the Investigator, subject is unlikely to be followed for the duration of the study.
13. Subject is unable or unwilling to comply with protocol requirements, including assessments, tests, and follow-up visits.
14. Subject has any other medical condition which in the opinion of the Investigator will make participation medically unsafe or interfere with the study results.

4 STUDY TREATMENT

4.1 INVESTIGATIONAL PRODUCT (DM199)

Active substance	DM199
Activity	Recombinant form of the endogenous serine protease protein, human tissue kallikrein-1 (rhKLK1)
Indication	Acute ischemic stroke
Strength	1 µg/kg intravenous; 3 µg/kg subcutaneous
Duration	Single 40-minute IV infusion; SC dose between 2 and 12 hours after start of infusion, then every 72 (± 2) hours after the first SC dose for the remainder of the 22-day treatment period (eight total SC doses)
Dosage form	IV infusion with subsequent SC injection
Manufacturer	Catalent

4.2 SELECTION OF DOSES IN THE STUDY

Based on biochemical analysis of DM199, 24 µg of DM199 represents a dose of 0.15 P-nitroaniline unit (PNAU). The selection of the doses for both IV and subcutaneous were selected based on results from previous completed clinical studies performed by DiaMedica.

4.3 CONDITIONS OF STORAGE AND ADMINISTRATION

The drug product is supplied in 1.5 mL vials containing 1 mL of 350 µg/mL DM199 in phosphate buffered saline and is to be stored at -20°C.

The appropriate dose of DM199 will be added to sterile normal saline and gently mixed per the instructions in the study pharmacy manual. Prior to administration, the solution in the vial will be carefully inspected visually for particulate matter and discoloration. If visible opaque particles, discoloration, or other foreign particulates are observed, the product will not be used.

Subcutaneous injection should be on the lower abdomen excluding the area two inches around the navel. Multiple injections may be required to deliver the total required dose and all of these injections should be administered subcutaneously in the lower abdomen successively. Different sites of the lower abdomen may be chosen to administer multiple injections. If condition of the skin prevents lower abdominal injection (e.g. successive SC injections for a condition unrelated to the study) alternative injection sites include the sides of the arms or the lateral side of the thighs. Details of study treatment administration, including dosing instructions, will be provided in the pharmacy manual.

Study treatment, including DM199 and placebo, must be stored in a secure area to prevent unauthorized access or use (21 CFR, Part 312, Subpart D). The Investigator is responsible for preventing use of the investigational product for non-investigational purposes.

4.4 MANAGEMENT OF STUDY TREATMENT TOXICITIES

Injection site reactions should be managed per standard of care. An alternative site should be chosen for future study treatments if local pain, tenderness, or swelling persists from a previous injection or other cause that has not resolved to Grade 0 or 1. Injection site reactions will be evaluated per section **6.4.2 Local (Injection Site) Adverse Event Intensity** and reported per section **6.3 Adverse Event Reporting**.

4.5 DOSE DELAYS AND MISSED DOSES

Study treatment must be withheld for any Grade ≥ 3 injection-site reaction or investigational product related AE. The delayed treatment may be resumed once the AE has resolved to Grade 0 or 1. However, subsequent injections must remain on schedule. A minimum of 72 ± 2 hours must elapse between injections. If the dose cannot be given within the scheduled window, then the injection must be missed. If ≥ 2 doses are missed due to AEs, then DM199 should be permanently discontinued.

If study treatment is delayed, all required testing that are scheduled to occur on dosing days must be repeated at the time treatment resumes. This includes: body weight, height, and BMI calculation; vital sign measurements; urine sample for KLK1 concentration, and clinical labs.

Study treatment should be permanently discontinued for any of the following reasons:

- Dosing is delayed for more than 6 days
- Any of the following adverse drug reactions (ADRs):
  - Any life-threatening (Grade 4) ADR
  - Severe (Grade 3) hypotension requiring sustained IV fluids replacement
  - Severe (Grade 3) hypoglycaemia
  - Severe (Grade 3) infusion-related reactions
  - Severe (Grade 3) ADR that recurs
- Observation of any other toxicities that in the opinion of the Investigator suggests that there may be a significant increase in either the incidence or severity of any events with study treatment. In this case, the Investigator will contact the Sponsor's Medical Monitor to conduct a review of all safety data.

Dose reduction of study treatment is not allowed.

Any study treatment delays or missed doses must be documented in the electronic case report form (eCRF) and in the subject's study chart, including a detailed reason for the change.

4.6 CONTRACEPTION

Female subjects of childbearing potential must use medically acceptable contraceptive measures to prevent pregnancy.

All women of childbearing potential (defined as sexually mature women who have had menses within the preceding 12 months and have not undergone hysterectomy, bilateral oophorectomy) must have a negative pregnancy test performed at screening.

Women of childbearing potential must agree not to attempt to become pregnant or undergo in vitro fertilization and, if participating in sexual activity that could lead to pregnancy, must use two reliable methods of contraception simultaneously while receiving protocol-specified medication and for 30 days after stopping the medication. Male heterosexual subjects participating in sexual activity must agree to use two reliable methods of contraception simultaneously during the study and for 30 days after stopping the medication if their partner is of childbearing potential.

A combination of two of the following methods must be used:

- Condoms (male or female)
- Diaphragm or cervical cap
- Intra uterine device
- Stable hormonal-based contraception

Women who are not of reproductive potential (who have been postmenopausal for more than 12 consecutive months or have undergone hysterectomy, bilateral oophorectomy) are not required to use contraception.

Male subjects and the partners of heterosexual female subjects will be required to use condoms to protect their partners from exposure to the study treatment, and prevent pregnancy.

4.7 PROHIBITED THERAPIES

Subjects are prohibited from taking angiotensin-converting-enzyme inhibitors (ACEi) during the study. They may be converted to another antihypertensive pharmacological treatment for continuity of care at the discretion of their physician.

4.8 ACCOUNTABILITY OF DRUG SUPPLY

The Sponsor will control the availability of the study treatment by shipping drug supplies only to qualified study Investigators who have Institutional Review Board/Ethics Committee (IRB/EC) approval to begin the clinical study. Drug supplies are not transferrable between Investigators unless prior written approval is obtained from the Sponsor.

All drug supplies, including investigational product and placebo, are to be used only for this protocol and not for any other purpose. Unless specifically instructed by DiaMedica, the Investigator must not destroy any drug labels, or any partly used or unused drug supply. The

Investigator must maintain an accurate record of the shipment and dispense of study treatments. Accountability will be reviewed by the monitor during site visits and at the completion of the trial. The Investigator will provide a copy of the accountability log to the monitor at the conclusion of the study. Any unused drug supplies must be returned to the Sponsor upon completion of site enrollment or as requested by Sponsor.

5 STUDY PARTICIPATION

Table 1: Schedule of Assessments

Assessment / Study Day	Screening/Baseline	Treatment Period *Day 1 and subsequent treatment days defined by treatment start time (not calendar days)*																				Follow-up Period		Early Withdrawal[12]
		Day 1*	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15	Day 16	Day 17	Day 18	Day 19	Day 20	Day 22	Day 56 (± 2 days)	Day 90 (± 2 days)	
Informed consent	X																								
Medical history/ Demographics	X																								
Inclusion/ Exclusion criteria	X																								
Randomization[1]	X																								
Previous and concomitant medication	X				X			X			X			X			X			X		X			(X)
Physical examination	X				X																	X	X	X	X
Weight, height, and BMI calculation[2]	X				X			X			X			X			X			X		X	X	X	X
Vital signs[3]	X	X			X			X			X			X			X			X		X	X	X	X
12-lead ECG[4]	X	X			X																	X			(X)
Urine pregnancy test[5]	X																						X		X

Assessment / Study Day	Screening/Baseline	Treatment Period *Day 1 and subsequent treatment days defined by treatment start time (not calendar days)*																				Follow-up Period		Early Withdrawal[12]
		Day 1*	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15	Day 16	Day 17	Day 18	Day 19	Day 20	Day 22	Day 56 (± 2 days)	Day 90 (± 2 days)	
Urine collection - urinary KLK1[6]	X				X									X								X		X	(X)
Clinical laboratory[7]	X	X			X																	X	X	X	X
Blood sampling forDM199 labs[8]	X		X																			X		X	X
DM199 or placebo: IV administration[9]		X																							
DM199 or placebo: SC administration[9]		X			X			X			X			X			X			X		X			
NIH Stroke Scale (NIHSS)	X[10]																					X		X	X
Barthel Index	X																					X		X	X
Modified Rankin Scale (MRS)	X																					X		X	X
Adverse event monitoring[11]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

1. A subject must meet all inclusion criteria and none of the exclusion criteria prior to randomization.
2. Weight, height, and BMI calculation required prior to first dosing. Subsequent height measurements not required, however, updated weights and BMI calculations are required on all dosing days (pre-dose) and days 56 and 90.
3. Vital sign measuremens include heart rate, systolic and diastolic blood pressure, respiratory rate, and body temperature. All measurements to be collected at baseline, on all dosing days 30 minutes post IV dose and 2 ± 0.25 hours post SC dose on days 1 and 4, post dose on days 7, 10, 13, 16, 19 and 22, and Days 56 and 90. Measurements will be captured after resting in a seated position for five minutes.
4. 12-lead ECG to occur at baseline, and post-dose on days 1, 4 and 22.
5. Completed at site for woment of child bearing potential only.
6. Urine collection for testsing of urinary KLK1 concentration to be obtained at baseline (anytime prior to first dose of study treatment), and pre-dose on days 4, 13, 22 and 90.

7. Clinical laboratory tests include clinical chemistry, hematology, urinalysis, and coagulation. Samples collected pre-dose at baseline and days 4, 22, 56, and 90. Day 1 samples collected post-IV dose but pre-SC dose.
8. DM199 labs include plasma concentration of DM199 and PD biomarkers at baseline and days 2, 22, and 90. Samples collected pre-dose.
9. Single 40 minute IV infusion of study treatment with DM199 or placebo to occur on day 1; subcutaneous (SC) dose between 2 and 12 hours after start of infusion, then every 72 (± 2) hours after the first SC dose for the remainder of the 22-day treatment period, for a total of eight SC doses.
10. For subjects undergoing reperfusion treatment prior to study enrollment, the NIHSS must occur at least 1 hour after treatment completion.
11. Adverse event (AE) collection will occur from time of consent to subject withdrawal. AEs will be captured during all hospitalizations and study visits or between visits as reported by the subject.
12. For any early subject withdrawal, every effort should be made to complete an early withdrawal visit. Assessments marked as (X) are only required if withdrawal occurs during the treatment period (day 1-22).

5.1 INFORMED CONSENT

Prior to enrolling in the study, potential subjects or their legally authorized representative (LAR) must be fully informed of the nature of the study, details of study procedures, anticipated benefits, and potential risks of study participation as required by applicable law. Before enrolling in the study or performing any study-specific procedures (including screening and baseline tests), provide the subject/LAR with a copy of the Informed Consent Form (ICF) and verbally review its content, allowing adequate time for questions. All information pertinent to the clinical study shall be provided in writing and in non-technical language that is understandable to the subject/LAR. Once the subject/LAR has read and understands the ICF, he/she will indicate his/her willingness to participate in the study by signing and dating the ICF. The ICF must also be signed and dated by the person obtaining informed consent. A copy of the signed informed consent will be given to the subject/LAR. The original consent documents will be retained in the Investigator Study File. The case history for each individual subject will include documentation that informed consent was obtained prior to participation in the study (21 CFR, Part 312.62).

The informed consent and documents must contain all the information required by 21 CFR, Part 50.25, ICH Guidance E6: *Good Clinical Practice: Consolidated Guidance,* and any additional elements required by local regulations. In addition to the document, the Investigator should provide oral information and answer questions from the subject.

5.2 ELIGIBILITY SCREENING

Figure 2 describes the process for screening patients for study eligibility.

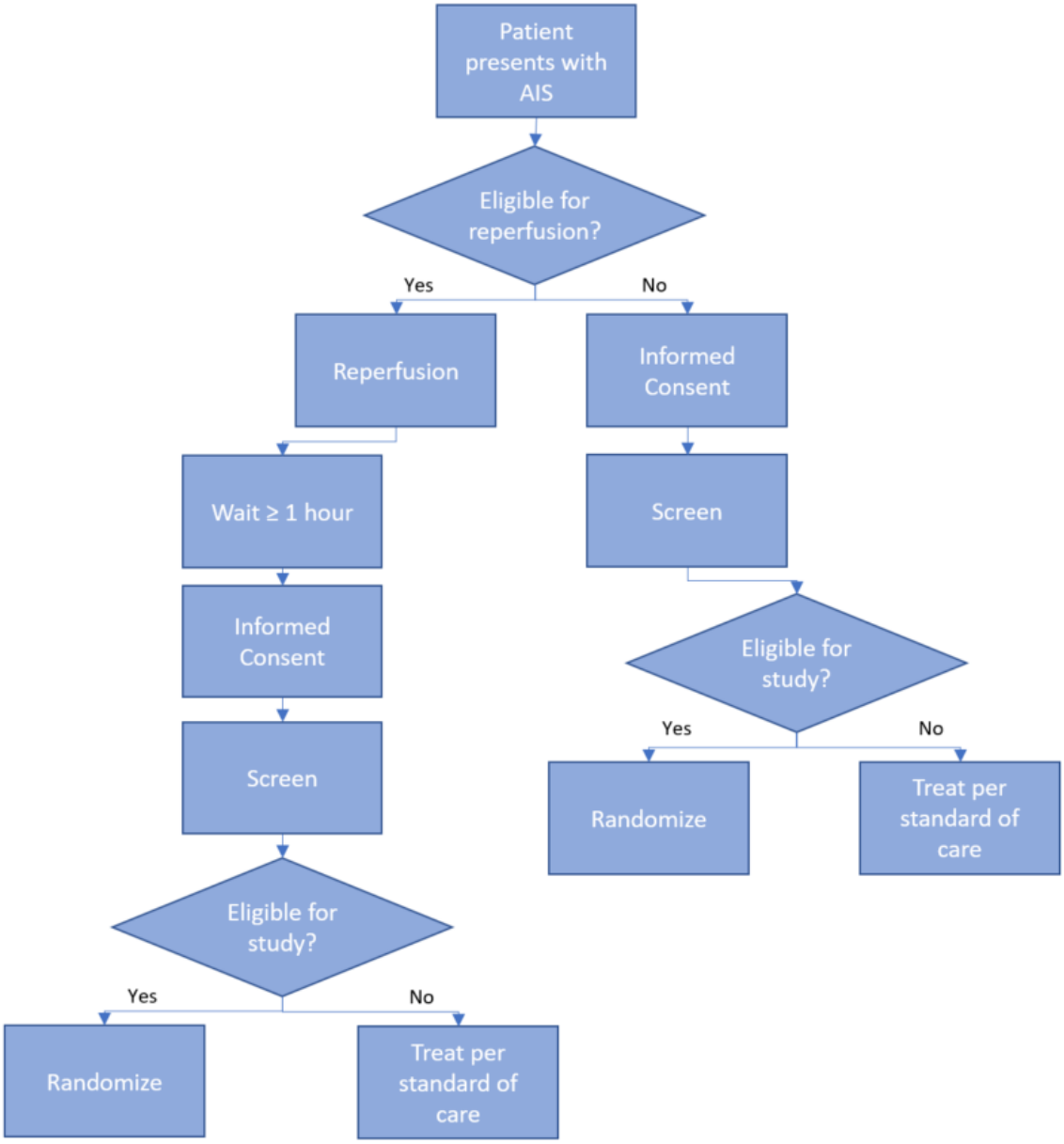

Figure 2. Screening Flowchart

Potential subjects presenting with symptoms of acute ischemic stroke should be assessed and monitored per institutional standard procedures. Those who are eligible for reperfusion (i.e. intravenous thrombolysis or mechanical thrombectomy) may be consented and screened for study eligibility after at least a one-hour period after treatment and must meet the same eligibility criteria for participation as subjects not being treated with reperfusion.

Potential subjects who are not eligible for reperfusion may be immediately consented and screened for study participation.

All eligible subjects, regardless of reperfusion status, must have a diagnosis of acute ischemic stroke and be enrolled ≤ 24 hours from time of stroke onset. After informed consent has been obtained, determine if the subject meets all the **Inclusion Criteria** and none of the **Exclusion Criteria** prior to randomization (enrollment).

5.3 RANDOMIZATION AND BLINDING

After it is confirmed that all eligibility criteria have been met, subjects will be randomized in a 1:1 fashion to receive DM199 or placebo. Subjects will be considered enrolled in the study after the informed consent form (ICF) has been signed, it is determined that he/she meets all eligibility criteria, and treatment assignment is obtained from the Interactive Web Response System (IWRS). No stratification will be incorporated into the master randomization schedule.

To minimize bias, the subject and Principal Investigator will be blinded to treatment assignment. All Sub-Investigators and other members of the study team will also remain blinded except for a designated unblinded pharmacist responsible for dispensing the assigned study treatment. The study team will remain blinded until all data is collected and database lock occurs. Until this time, the unblinding may only occur via authorized use of the IWRS only if knowledge of treatment is needed to manage a subject's condition (refer to **6.5.2 Safety Unblinding**).

5.4 PHYSICAL EXAMINATION

A complete physical examination per institutional standards is required at screening and days 4, 22, 56, and 90.

Note: Refer to the Schedule of Assessments and sections below for details on protocol requirements for height, weight, and vital sign measurements.

5.5 BODY WEIGHT AND HEIGHT

Body weight, height, and body mass index (BMI) will be captured at screening. Updated weight and BMI calculations will be obtained prior to study treatment administration on all IV and SC dosing days, and on days 56 and 90.

5.6 VITAL SIGNS

Vital signs will be obtained at the following timepoints:

- Baseline
- Day 1: 30 minutes post IV dose
- Days 1 and 4: 2 ± 0.25 hours post SC dose
- Remaining dosing days (7, 10, 13, 16, 19, and 22): Post dose
- Day 56
- Day 90

Required vital sign measurements include heart rate, systolic and diastolic blood pressure, respiratory rate, and body temperature. Vital signs will be measured after resting in a seated position for five minutes.

5.7 ELECTROCARDIOGRAM

12-lead ECG measurements will be obtained at baseline, and post dose on days 1**,** 4, and 22 after the subject is supine for at least three minutes.

5.8 URINE PREGNANCY TEST

A urine pregnancy test is required to confirm eligibility prior to enrollment for any woman of child bearing potential (refer to **4.6 Contraception**). All urine pregnancy tests will be collected pre-dose, and results recorded prior to dosing.

5.9 CENTRAL LABORATORY ASSESSMENTS

5.9.1 Clinical Laboratory

Pre-dose sample collection for Chemistry, Hematology, Urinalysis, and Coagulation will be collected, stored, and shipped according to the Central Laboratory Manual at baseline and days 4, 22, 56, and 90. Day 1 samples will be collected post-IV dose, but pre-SC dose. Urine pregnancy test is to be confirmed at the site (refer to **5.8 Urine Pregnancy Test**). **Table 2** describes the parameters to be measured.

Table 2: Clinical Laboratory Collection Parameters

Analysis	**Parameters**
Clinical Chemistry (serum quantitatively)	Total bilirubin, alkaline phosphatase (ALP), gamma glutamyl transferase (gamma-GT), AST, ALT, lactate dehydrogenase (LDH), creatinine, urea, total protein, random glucose, inorganic phosphate, sodium, potassium, calcium, chloride, magnesium, bicarbonate, Blood Urea Nitrogen (BUN), creatinine
Hematology (blood quantitatively)	Leukocytes, hemoglobin, hematocrit, platelets, **partial automated differentiation:** lymphocytes, monocytes, eosinophils, basophils, neutrophils, **red blood cell parameters:** mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cell distribution width (RDW), red blood cell (RBC) count, HbA1c
Urinalysis (urine qualitatively)	Blood, specific gravity, pH, leukocytes, nitrates, urobilinogen, ketones, glucose, protein

Coagulation	Prothrombin time (PT) (seconds and International Normalized Ratio (INR)), activated partial thromboplastin time (aPTT)
Urine Pregnancy Test	hCG (For women of child bearing potential only)

In the event of unexplained or unexpected clinical laboratory test values, the test(s) will be repeated at appropriate intervals and followed up until the results return to baseline and/or to a level deemed acceptable by the Investigator (or medically qualified designee) and the Sponsor's Medical Monitor (or his/her designated representative), or an adequate explanation for the abnormality is found. The central laboratory will clearly mark all laboratory test values that are outside the normal range, and the Investigator will indicate which of these are clinically significant. Clinically significant laboratory test results will then be recorded as AEs and the relationship to the treatment will be indicated (refer to **6. Safety Oversight**).

5.9.2 DM199 Laboratory

Pre-dose sample collection for plasma concentration of DM199 and pharmacodynamic biomarkers will be collected, stored, and shipped according to the Central Laboratory Manual at baseline and on days 2, 22, and 90. Urinary KLK1 concentration will be collected, stored, and shipped according to the Central Laboratory Manual at baseline and days 4, 13, 22 and 90. **Table 3** describes the parameters to be measured.

Table 3: DM199 Laboratory Collection Parameters

Analysis	**Parameters**
Pharmacokinetic biomarkers	Plasma concentration of DM199
Diagnostic biomarkers	Urinary concentration of KLK1
Pharmacodynamic biomarkers	CRP, MMP-9, VEGF, Nitric Oxide [NOx], Prostaglandins [E2], DM199 ADA, and tPA

5.10 INTRAVENOUS TREATMENT ADMINISTRATION

After it is confirmed that all eligibility criteria have been met, study treatment has been assigned through the randomization system, and all pre-dosing (baseline) assessments are complete, a single 40-minute IV infusion of DM199 or placebo will be administered on day 1. Refer to **4. Study Treatment** and the pharmacy manual for information on proper storage, dosing, and administration.

5.11 SUBCUTANEOUS TREATMENT ADMINISTRATION

A single subcutaneous dose of DM199 or placebo will be administered between 2 and 12 hours after the start time of the IV dose. Additional doses will then occur every 72 (± 2) hours after the first SC dose for the duration of the 22-day treatment period. In total, eight SC doses must be administered. Refer to **4. Study Treatment** and the pharmacy manual for information on proper storage, dosing, and administration.

Note: The treatment period (days 1-22) are defined by the time of the IV dose of study treatment rather than calendar days. Therefore, the first SC dose administered between 2 and 12 hours post IV may occur within the same calendar day or the day after. Caution should be taken to adjust all subsequent SC doses based on the required 72 (± 2) window and required testing that is to occur on dosing days, including: body weight, height, and BMI calculation; vital sign measurements; urine sample for KLK1 concentration, and clinical labs.

5.12 NIH STROKE SCALE

For subjects undergoing reperfusion treatment prior to study enrollment the NIH stroke scales (NIHSS) must occur at least one hour after treatment. The scale will also be administered at days 22 and 90.

A NIHSS of ≥ 6 and ≤ 25 at enrollment is required for study inclusion.

The NIHSS will be administered by the Investigator or a designated and trained member of the study team.

5.13 BARTHEL INDEX

The Barthel Index will be administered at baseline (any time prior to first dose of study treatment), days 22 and 90 by the Investigator or a designated and trained member of the study team.

5.14 MODIFIED RANKIN SCALE

The Modified Rankin Scale (MRS) be administered at baseline (any time prior to first dose of study treatment), days 22 and 90 by the Investigator or a designated and trained member of the study team.

5.15 SUBJECT WITHDRAWAL

Participation in the study is strictly voluntary. A subject has the right to withdraw from the study at any time for any reason.

The Investigator has the right to terminate the participation of a subject for any of the following reasons:

- The need to take medication which may interfere with study assessments

- Subject's non-compliance with study requirements
- Difficulties in obtaining blood samples
- Severe or serious adverse events (SAEs), or for any other reason relating to the subject's safety or integrity of the study data

If a subject is withdrawn from the study, the Medical Monitor will be informed immediately. If there is a medical reason for withdrawal, the subject will remain under the supervision of the Investigator until satisfactory health has returned.

The reasons for withdrawal will be recorded on the eCRF and included in the final clinical study report, along with any AEs and any necessary medical treatment. In the event that a subject is discontinued from the study due to a significant AE, SAE, or abnormal laboratory result (refer to **6.2.4 Clinical Laboratory Abnormalities and Other Abnormal Assessments**), the Investigator or medically trained designee will evaluate the urgency of the event. If the situation warrants, the Investigator or medically trained designee will take appropriate diagnostic and therapeutic measures. If the situation is not an immediate emergency, the Investigator, or medically trained designee, will attempt to contact DiaMedica's assigned Medical Monitor for consultation. No medical help, diagnosis, or advice will be withheld from the subject due to an inability to contact the Medical Monitor. The subject will be encouraged to remain available for follow-up medical monitoring. The Sponsor will be notified as soon as possible of any early withdrawals.

Every effort should be made to complete an Early Withdrawal Visit for a subject that withdrawals from the study prior to completing all study requirements. Refer to the **Table 1: Schedule of Assessments** for a description of testing that should occur during the Early Withdrawal Visit. The reasons for early withdrawal will be recorded on the Early Withdrawal eCRF.

6 SAFETY OVERSIGHT

6.1 DATA SAFETY MONITORING BOARD (DSMB)

An independent DSMB will be formed to advise the Sponsor regarding the continuing safety of the trial subjects and those yet to be recruited to the trial, as well as the continuing validity and scientific merit of the trial. At a minimum, the DSMB will meet semi-annually. The committee will be composed of three clinician experts in stroke treatment and a biostatistician who have no affiliation with the clinical study. A DSMB charter will be developed and agreed upon by the Sponsor and DSMB members and will describe the specific functions of the DSMB. The primary responsibilities of the DSMB are to:

- Review and validate the subject sample (i.e., review inclusion/exclusion deviations and other protocol deviations)
- Provide oversight for issues affecting general subject welfare

- Establish study termination guideline criteria
- Recommend premature study termination

At any time during the course of the study, the DSMB may offer opinions or make formal recommendations concerning aspects of the study that impact subject safety (e.g., safety related protocol changes or input regarding adverse event rates associated with the investigational product). Any DSMB recommendations for study modification or termination due to concerns over subject safety or issues relating to data monitoring or quality control will be submitted in writing to the National PI and Sponsor for consideration and final decision.

6.2 DEFINITIONS

6.2.1 Adverse Event

An adverse event (AE) is any untoward medical occurrence in a subject or clinical investigation subject administered a pharmaceutical product and which does not necessarily have to have a causal relationship with the treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational/experimental) product, whether or not related to the product (Refer to International Conference on Harmonization [ICH] E2A: Clinical Safety Data Management: Definitions and Standards for Expedited Reporting, 27 October 1994).

Treatment-emergent AEs will be defined as AEs with an onset after administration of the study treatment, or when a preexisting medical condition increases in severity or frequency after study treatment administration.

Adverse Events (AEs) will not include:

- A medical or surgical procedure such as surgery, endoscopy, tooth extraction, or transfusion (although the condition that leads to the procedure may be an AE)
- A pre-existing disease or condition present at the start of the study that does not worsen during the study
- Any situation where an untoward medical occurrence has not occurred (for example, hospitalizations for cosmetic elective surgery or social admissions)
- An overdose of either the investigational product or a concurrent medication without any resulting signs or symptoms

6.2.2 Serious Adverse Event

A Serious Adverse Event (SAE) is any AE that:

- Results in death;[1]
- Is life-threatening;[2]
- Requires inpatient hospitalization or prolongation of an existing hospitalization;[3]
- Results in persistent or significant disability/incapacity;

- Is a congenital anomaly/birth defect, or;
- Is a medically important event.[4]

1. The death of a subject enrolled in a study is per se not an event, but an outcome. Any event resulting in a fatal outcome must be fully documented and reported, regardless of the causality relationship to the investigational product.
2. The term life threatening refers to an AE in which the subject was at risk of death at the time of the event. It does not refer to an event which hypothetically might have caused death if it was more severe.
3. Hospitalization is defined as 24 hours in a hospital or an overnight stay. An elective hospital admission to treat a condition present before exposure to the test drug, or a hospital admission for a diagnostic evaluation of an AE, does not qualify the condition or event as an SAE. Further, an overnight stay in the hospital that is only due to transportation, organization, or accommodation problems and without medical background does not need to be considered an SAE.
4. Medical and scientific judgment should be exercised in deciding whether other situations should be considered serious such as important medical events that may not be immediately life threatening or result in death or hospitalization but might jeopardize the subject or might require medical or surgical intervention to prevent one of the other serious outcomes listed in the above definition. These should also be considered serious. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias, or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

6.2.3 Adverse Drug Reaction (ADR)

In the pre-approval clinical experience with a new medicinal product or its new usage, particularly as the therapeutic doses may not be established, all noxious and unintended responses to a medicinal product related to any dose should be considered adverse drug reactions. The phrase "responses to a medicinal product" means that a causal relationship between a medicinal product and an AE is at least a reasonable possibility, i.e. the relationship cannot be ruled out.

6.2.4 Clinical Laboratory Abnormalities and Other Abnormal Assessments

Abnormal laboratory findings (e.g., clinical chemistry, hematology, coagulation and urinalysis) or other abnormal assessments (e.g., ECGs, vital signs) that are judged by the Investigator (or medically qualified designee) at the site as clinically significant or result in clinical sequelae will be recorded as AEs if they meet the definition of an AE (refer to **6.2.1 Adverse Event**). Clinically significant abnormal laboratory findings or other abnormal assessments that are detected during the study or are present at baseline and significantly worsen following the start of the study will be reported as AEs. The Investigator will exercise his or her medical and scientific judgment in deciding whether an abnormal laboratory finding or other abnormal assessment is clinically significant.

6.2.5 Serious Adverse Events Related to Study Participation

A SAE considered related to study participation (e.g., procedures, invasive tests, a change in existing therapy), even if it occurs during the pre- or post-treatment period, will be reported.

6.3 ADVERSE EVENT REPORTING

6.3.1 Reporting Period

Subjects will be evaluated for safety if they have received any treatment. Any preexisting conditions or signs and/or symptoms present in a subject prior to the start of the study (i.e., before informed consent) should be recorded as medical/surgical history. All AEs occurring after informed consent and on or before the final visit must be reported as AEs; only AEs that occur post-dose will be considered treatment-emergent. All AEs must be recorded irrespective of whether they are considered drug related.

6.3.2 Adverse Event Collection

All AEs reported by the subject or noted in the medical record during the course of the study will be evaluated. At each encounter, subjects will be asked non-leading questions to determine the occurrence of AEs. AEs will be elicited using a standard non-leading question such as "How did you feel since last visit?" In addition, any signs or symptoms will be observed.

Adverse events may be collected from:

- The subject's positive response to questions about their health
- Symptoms spontaneously reported by the subject
- Clinically relevant changes and abnormalities observed by the Investigator (e.g. local and systemic tolerability, laboratory measurements, results of physical examinations)

When an AE occurs, it is the responsibility of the Investigator to review all documentation relative to the event, including, but not limited to progress notes, laboratory, and diagnostic reports. The Investigator will then ensure that all relevant information regarding an AE is expediently recorded in accordance with the eCRF guidelines, including proper coding of the event term per the Medical Dictionary for Regulatory Activities (MedDRA). Investigators should be mindful of expedited reporting requirements for SAEs (refer to 6.5 Expedited Reporting of Serious Adverse Events). The Investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. In all cases, when available, the diagnosis should be reported as the AE and not the individual signs/symptoms. It is not acceptable for the Investigator to send photocopies of the subject's medical records to the Sponsor in lieu of completion of the appropriate AE eCRF pages.

The Investigator or designee will record all AEs in the source document as well as in each subject's eCRF with information about:

- Adverse event and relevant clinical findings
- Time/date of onset
- Time/date of recovery or current status of adverse event

- Intensity/severity
- Action taken on study treatment
- Other action taken to treat the event
- Relation to study treatment
- Seriousness of the AE

6.3.3 Adverse Event Follow-up

After the initial AE report, the Investigator is required to proactively follow each subject and provide further information to the Sponsor on the subject's condition as deemed appropriate. All AEs will be followed until resolution, until the condition stabilizes, until the event is otherwise explained, or until the subject is lost to follow-up. Once resolved, the appropriate AE eCRF page and SAE report form (if applicable) will be updated. The Investigator will ensure that follow-up includes any supplemental investigations as may be indicated to elucidate the nature and/or causality of the AE. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals. In the event of a fatal outcome in an SAE, the Investigator will attempt to obtain postmortem findings, including histopathology and provide all additional information in a follow up SAE report. New or updated information will be recorded on a new SAE report form marked as a follow-up with the appropriate follow-up number added to the report. The follow-up report will be signed and dated by the Investigator or medically qualified designee.

6.3.4 Pregnancy Reporting

If a subject inadvertently becomes pregnant during the study the pregnancy will be reported to the Sponsor via the Pregnancy eCRF. If the pregnancy meets the definition of a SAE information will be reported using the SAE Report Form submitted within 24 hours of knowledge of the event. Study treatment will immediately cease upon learning of a subject pregnancy. The Investigator or designee will make every effort to obtain permission to follow the outcome of the pregnancy and report the condition of the fetus and newborn to the Sponsor's Medical Monitor. The Investigator or designee will attempt to contact the subject at least monthly and document the subject's status until the pregnancy has been completed or terminated. The pregnancy outcome will be reported to the Sponsor's Medical Monitor (or designee) without delay, or within 24 hours if the outcome is a serious adverse event (e.g., death, abortion, congenital anomaly, or other disabling or life-threatening complication to the mother or newborn).

If a male subject impregnates his female partner the pregnancy should be reported to the Sponsor's Medical Monitor and followed as described above.

6.3.5 Post-study Adverse Events

A post-study AE is defined as any event that occurs outside of the AE reporting period defined in **6.3.1 Reporting Period.**

Investigators are not obligated to actively seek AEs in former study subjects. However, if the Investigator learns of any SAE, including death, at any time after a subject has been discharged from the study, and he/she considers the event reasonably related to the investigational product, the Investigator will promptly notify the Sponsor.

6.4 EVALUATION OF ADVERSE EVENTS

6.4.1 Assessment of Intensity

Adverse events and other symptoms will be graded according to National Cancer Institute's Common Toxicity Criteria for Adverse Events version 4.03 (CTCAE v4.03).

The Investigator or medically qualified designee will make an assessment of intensity for each AE reported during the study. The assessment will be based on the Investigator's (or medically qualified designee's) clinical judgment. The intensity should be assigned to one of the following categories:

Table 4: CTCAE Grading

Grade	Clinical Description of Severity
0	No Change from Normal or Reference Range *(This grade is not included in the Version 4.0 document but may be used in certain circumstances.)*
1	MILD Adverse Event Asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
2	MODERATE Adverse Event Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living (ADL)*.
3	SEVERE Adverse Event Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL**.
4	LIFE-THREATENING OR DISABLING Adverse Event Life-threatening consequences; urgent intervention indicated.
5	DEATH RELATED TO Adverse Event

**Instrumental ADL refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.*
***Self-care ADL refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.*

An AE that is assessed as severe should not be confused with a SAE. Severity is a category utilized for rating the intensity of an event; and both AEs and SAEs can be assessed as severe.

If an AE worsens in intensity, it should be recorded as a new AE. If an AE gets milder in intensity, it continues as the first report until the subject is recovered.

6.4.2 Local (Injection Site) Adverse Event Intensity

Intensity of the following local injection site AEs should be assessed as described in **Table 5** adapted from the National Cancer Institute's Common Toxicity Criteria for Adverse Events version 4.03 (CTCAE v4.03).

Table 5: Grading of Local (Injection Site) Adverse Event Intensity

Local Reaction to Injectable Product	Absent (Grade 0)	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Pain	Absent	Mild pain	Moderate pain; limiting instrumental ADL	Severe pain; limiting self-care ADL	Emergency room (ER) visit or hospitalization
Tenderness	Absent	Mild discomfort to touch	Discomfort with movement	Significant discomfort at rest	ER visit or hospitalization
Pruritus	Absent	Mild or localized	Intense or widespread; intermittent	Intense or widespread; constant	ER visit or hospitalization
Urticaria (hives, welts, wheals)	Absent	Urticarial lesions covering <10% Body Surface Area (BSA)	Urticarial lesions covering 10 - 30% BSA	Urticarial lesions covering >30% BSA	ER visit or hospitalization
Erythema/ Redness *	Absent	Target lesions covering	Target lesions covering 10 - 30% BSA and associated with skin tenderness	Target lesions covering >30% BSA and associated with oral or genital erosions	Target lesions covering >30% BSA; associated with fluid or electrolyte abnormalities; ICU care or burn unit indicated
Induration/ Swelling**	Absent	Mild induration, able to move skin parallel to plane (sliding) and perpendicular to skin (pinching up)	Moderate induration, able to slide skin, unable to pinch skin; limiting instrumental ADL	Severe induration, unable to slide or pinch skin; limiting joint movement or orifice (e.g., mouth, anus); limiting self-care ADL	Generalized; associated with signs or symptoms of impaired breathing or feeding

* In addition to grading the measured local reaction at the greatest single diameter, the measurement should be recorded as a continuous variable.

** Induration/Swelling should be evaluated and graded using the functional scale as well as the actual measurement.

6.4.3 Assessment of Causality

The Investigator or medically qualified designee is obligated to assess the relationship between investigational product and the occurrence of each AE. The Investigator or medically qualified designee will use clinical judgment to determine the relationship. Alternative causes, such as natural history of the underlying diseases, concomitant therapy, other risk factors, and the

temporal relationship of the event to the investigational product will be considered and investigated. The Investigator or medically qualified designee will also consult the Investigator's Brochure in the determination of his/her assessment.

There may be situations when a SAE has occurred and the Investigator has minimal information to include in the initial SAE report. However, it is very important that the Investigator (or medically qualified designee) always make an assessment of causality for every event prior to transmission of the SAE report form (refer to **6.5.1 Completion and Transmission of SAE Reports**). The Investigator or medically qualified designee may change his/her opinion of causality in light of follow-up information and amend the SAE report form accordingly. The causality assessment is one of the criteria used when determining global regulatory reporting requirements.

The Investigator will provide the assessment of causality utilizing one of four possible categories: Unrelated, Unlikely, Possible, and Probable.

- **Unrelated:** Clearly and incontrovertibly due to extraneous causes, and does not meet criteria listed under unlikely, possible or probable.
- **Unlikely:** Does not follow a reasonable temporal sequence from administration. May have been produced by the subject's clinical state or by environmental factors or other therapies administered.
- **Possible:** Follows as reasonable temporal sequence from administration. May have been produced by the subject's clinical state or by environmental factors or other therapies administered.
- **Probable:** Clear-cut temporal association with improvement on cessation of test drug or reduction in dose. Follows a known pattern of response to test drug.

6.5 EXPEDITED REPORTING OF SERIOUS ADVERSE EVENTS

AEs meeting serious criteria MUST be reported promptly to the designated Sponsor CRO, and the local Institutional Review Board/Ethics Committee (IRB/EC). Prompt notification of SAEs by the Investigator is essential so that the Sponsor may comply with its global regulatory obligations.

6.5.1 Completion and Transmission of SAE Reports

Once an Investigator becomes aware that a SAE has occurred in a study subject, she/he will report the information on the SAE Report Form to the designated Sponsor contract research organization (CRO) within 24 hours. The SAE Report Form will always be completed as thoroughly as possible with all available details of the event and signed by the Investigator or medically qualified designee. If the Investigator does not have all information regarding the SAE, he/she will not wait to receive additional information before reporting the event but rather will update the SAE Report Form when additional information is received.

The Investigator or medically qualified designee will always provide an assessment of causality at the time of the initial report as described in **6.4.3 Assessment of Causality**.

Email transmission of the SAE Report Form is the preferred method to transmit this information to the designated Sponsor CRO. In rare circumstances notification by telephone is acceptable, however, notification via the telephone does not replace the need for the Investigator or medically qualified designee to complete and sign the SAE report form within the outlined time frame.

The Sponsor will provide a list of project contacts for SAE reporting including telephone, email, and fax numbers. Any event that in the opinion of the Investigator may be of immediate or potential concern for the subject's health or well-being will be reported to the Sponsor's Medical Monitor emergency contact.

6.5.2 Safety Unblinding

If knowledge of treatment assignment is needed to manage a subject's condition, as in the case of an immediately reportable SAE, the Principal Investigator or designee will contact the Medical Monitor who, in consultation with the Sponsor, will grant permission to unblind the subject in accordance with the Unblinding Plan. The only exception is when a medical emergency exists and authorization cannot be immediately obtained.

If unblinding occurs, the date and reason for unblinding (but not the subject's treatment assignment) will be documented. Any subject who requires unblinding will be discontinued from further study treatment and an Early Withdrawal Visit will be completed. Subjects will not be routinely unblinded in the case of an AE.

6.5.3 Serious Adverse Event Reports to the IRB/EC

The Investigator, or responsible person according to local requirements, will comply with the applicable local regulatory requirements related to the reporting of SAEs to regulatory authorities and the IRB/EC.

7 DATA COLLECTION AND MANAGEMENT

7.1 DATA MANAGEMENT

All required clinical data for this trial will be collected in web-based standardized electronic case report forms (eCRFs). FDA 21 CFR, Part 11 will be followed as well as other applicable legislation on the handling of electronic data. Subject personal information will be pseudonymized.

A Data Management Plan (DMP) will be developed outlining the procedures used for data review, database cleaning and issuing and resolving data queries. Procedures for validations and data storage will also be contained within the DMP.

Investigators and designated staff will receive training on eCRF completion and use of the electronic data capture (EDC) system prior to use.

7.2 SUBJECT IDENTIFICATION

After obtaining oral and written informed consent by the subject or his/her legally authorized representative, subjects will be screened according to the inclusion and exclusion criteria. If all eligibility criteria have been met, subjects will be randomized and enrolled into the study. Enrolled subjects will be assigned a unique subject number that will be used to identify them on source documents, eCRFs, and reports. Those who do not meet eligibility criteria or withdraw consent for any reason before completing all screening evaluations will be considered screen failures. Screen failures will not be entered in the eCRFs.

The Investigator or authorized designee at each site will keep a screening and enrollment log of all subjects screened and enrolled to document subject numbers and reasons for screen failures or withdrawals.

7.3 CASE REPORT FORMS

The Investigator is responsible for ensuring the accuracy and completeness of all study documentation. All protocol-required information collected during the study must be entered by the Investigator, or designated representative, in the eCRF. Each eCRF should be completed as soon as possible after data are collected, preferably on the same day the subject is seen for an examination, treatment, or any other study procedure and at the latest before the next monitoring visit. An explanation should be given for all missing data.

The completed eCRFs must be reviewed and signed by the Investigator named in the clinical study protocol.

7.4 SOURCE DOCUMENTATION

Original or certified copies of all relevant clinical findings, observations, and other activities throughout the clinical investigation must be recorded and maintained in the medical file of each enrolled subject. No source documentation will be recorded directly on the eCRF. Source documents may include, but are not limited to, admission/discharge records, lab and imaging reports, demographic records, and clinic visit records. A source data location list will be prepared prior to study start. This list will be filed in both the Trial Master File and the Investigator Study File and updated as necessary.

The Investigator will permit study-related monitoring, audits, IRB/EC review and authority inspections (refer to **12. Study Monitoring and Auditing**) by allowing direct access to all study records. In case of electronic source data, access must be allowed or dated print-outs must be available prior to the monitoring visits. Print-outs will not be limited to the treatment data only, but will include all available data related to the identified subject(s).

7.4.1 Source Documentation Reporting Requirements

Images and corresponding reports for any brain imaging completed as part of standard of care treatment for any enrolled subject throughout the duration of the study (i.e. initial presentation through withdrawal) will be submitted to the CRO. In addition, it may be necessary from time to time to submit other source documents to the CRO or Sponsor to allow for timely safety discussions (i.e. expedited reporting of SAEs).

All source documents submitted to the Sponsor or its representatives, including imaging and reports, must be de-identified according to local and federal confidentially requirements and identified by the subject number.

8 STATISTICAL CONSIDERATIONS

8.1 STATISTICAL DESIGN AND SAMPLE SIZE

8.1.1 General Design

This is a randomized, double-blind, placebo-controlled Phase II, multi-center study of DM199. Subjects presenting with acute ischemic stroke will be randomized 1:1 to placebo or DM199 administered by a single intravenous (IV) dose followed by subsequent subcutaneous (SC) doses.

8.1.2 Sample Size

No formal sample size estimation was performed. Up to 66 subjects will be enrolled to achieve approximately 60 completed subjects, based on an estimated drop-out rate of 10%. Subjects will be randomized 1:1 to receive DM199 or placebo, resulting in 33 enrolled subjects per treatment group. A sample size of 30 completed subjects in each treatment group is considered adequate to evaluate the safety and tolerability of DM199. Should a subject drop out, the drop out subject may be replaced by a subject assigned to the same treatment arm as the drop out subject.

8.1.3 Analysis Populations

There are three analysis populations: Safety, modified Intention-to-treat (mITT), and Pharmacodynamics (PD).

8.1.3.1 Safety Population

All randomized subjects who receive any amount of study treatment will be included in the Safety Population. Subjects will be summarized according to the treatment they actually received. Demographic, baseline characteristics, plasma concentrations of DM199, and Safety analysis will be conducted using the Safety Population.

8.1.3.2 Modified Intention-to-Treat (mITT) Population

All randomized subjects who receive at least one dose of study treatment and who have at least one post-dose efficacy assessment will be included in the mITT population. Subjects will be

summarized according to the treatment they are randomized to receive. Efficacy analysis will be conducted using the mITT population.

8.1.3.3 Pharmacodynamic Population

Randomized subjects who receive study medication and have sufficient plasma concentration for analysis of the PD endpoints will be included in the pharmacodynamic analysis. Subjects with protocol violations will be assessed on a subject-by-subject basis for inclusion in the PD population. PD analysis will be conducted using the PD population.

Any additional analysis populations will be detailed in the Statistical Analysis Plan (SAP).

8.1.4 Interim Analyses

No interim analyses are planned for this study.

8.2 STUDY ENDPOINTS

8.2.1 Primary Endpoints

Primary endpoints include:

1. Safety:
    - Incidence, severity and causality of adverse events (AE) and serious adverse events (SAE)
    - Physical examination changes from baseline (days 4, 22, 56, and 90)
    - Vital sign changes from baseline (resting heart rate, systolic/diastolic blood pressure, respiratory rate, and temperature) (days 1, 4, 7, 10, 13, 16, 19, 22, 56, and 90)
    - Hematology and chemistry parameter changes from baseline (days 4, 22, 56, and 90)
    - 12 lead ECG changes from baseline (days 1, 4, and 22)
2. Tolerability as assessed by incidence and severity of injection site adverse events.

8.2.2 Secondary Endpoints

The secondary endpoints of this study are to evaluate:

1. Efficacy as assessed by:
    - NIH Stroke Scale (days 22 and 90)
    - Barthel Index (days 22 and 90)
    - Modified Rankin Scale (days 22and 90)
2. Plasma concentration of DM199 (days 2, 22, and 90)

3. Pharmacodynamics as assessed by:
    - Plasma C-reactive protein (CRP) (days 2, 22, and 90)
    - Plasma MMP-9 (days 2, 22, and 90)
    - Plasma VEGF (days 2, 22, and 90)
    - Plasma DM199 ADA (days 2, 22, and 90)

8.2.3 Exploratory Endpoints

Exploratory endpoints include:

1. Urinary KLK1 concentration (days 4, 13, 22 and 90)
2. Pharmacodynamics as assessed by:
    - Plasma tPA (days 2, 22, and 90)
    - Plasma Nitric Oxide as NOx (days 2, 22, and 90)
    - Plasma Prostaglandins (E2) (days 2, 22, and 90)

8.3 STATISTICAL METHODS

Data will be handled and processed according to the sponsor's representative (Novotech (Australia) Pty Ltd) Standard Operating Procedures (SOPs), which are written based on the principles of GCP.

All data collected on the eCRFs and results of central laboratory analysis will be presented in the data listings. All summaries will present the data by treatment group and overall (total subjects), as applicable.

Statistical methods will be primarily descriptive in nature. Each treatment group will be summarized separately. No formal statistical comparisons of DM199 versus placebo will be made.

The following statistical approaches will be taken:

- Continuous variables: Descriptive statistics will include the number of non-missing values (N), mean, standard deviation (SD), median, minimum, maximum. Additionally, for plasma concentrations of DM199, the coefficient of variation (CV%), geometric mean and geometric coefficient of variation (geo CV%) values may also be presented.
- Categorical variables: Descriptive statistics will include frequency counts and percentages per category.
- Imputation: No missing data will be imputed generally. The handling and presentation of plasma concentrations of DM199 below the limit of quantification (BLQ) will be discussed in the Statistical Analysis Plan.

- Confidence intervals (CIs): CIs will be two-sided and will use 95% confidence levels unless specified otherwise.
- Baseline: Baseline values will be defined as the last non-missing observation for each subject prior to the first dosing of study medication (i.e. start of infusion on Day 1).

Detailed statistical methods will be provided in a separate Statistical Analysis Plan (SAP) prior to data base lock.

8.3.1 Baseline Summaries

Demographic and baseline characteristics will be summarized descriptively by treatment group.

8.3.2 Safety

Safety endpoints will be analyzed using the Safety population.

8.3.2.1 Adverse Events/Serious Adverse Events

Adverse events (AEs) will be coded using the Medical Dictionary for Regulatory Activities (MedDRA®) and data will be summarized by System Organ Class (SOC) and Preferred Term (PT). The number and percent of subjects reporting each AE will be summarized for each treatment group and overall. A subject with two or more AEs within the same level of summarization (i.e., SOC or PT) will be counted only once in that level using the most severe (i.e., highest severity/grade) event or most related (for the relationship to study treatment table). Percentages will be based on the number of subjects in the Safety Population within each treatment group. The number of AEs reported will also be presented.

Treatment-emergent AEs (TEAEs) are defined as pre-treatment existing conditions that worsen after study treatment administration, or events that occur during the course of the study during or after administration of study treatment. Only TEAEs will be included in the AE summary tables which will present data by treatment group. In the case of a missing AE start date or stop date, the most conservative approach will be followed.

An overall summary of AEs will be produced including the number of TEAEs; the number and % of subjects reporting at least one: TEAE, serious TEAE, grade 3 or higher TEAE, TEAE related to study treatment (possible, probable), serious TEAE related to study treatment, grade 3 or higher TEAE related to the study treatment, TEAEs by maximum severity and TEAEs by maximum relationship, as appropriate.

Additional AE tables will be generated as follows, as appropriate:

- Overall TEAEs by SOC and PT
- TEAEs by Maximum Severity
- TEAEs by Maximum Relationship to Study Treatment
- Treatment Emergent SAEs

- TEAEs with Grade 3 or Higher
- Study Treatment Related TEAEs by SOC and PT
- Study Treatment Related TEAEs by Maximum Severity

A by-subject AE data listing, including verbatim term, MedDRA SOC and PT, severity, outcome and relationship to study treatment, will be provided. A separate listing will be generated for SAEs.

8.3.2.2 Physical Examination Findings

Physical examination data for all time points assessed will be summarized, including Investigator assessment of clinical significance for abnormal findings. Shift tables from baseline to days 4, 22, 56 and 90 may also be generated, as applicable.

8.3.2.3 Vital Signs

All vital sign parameters will be summarized using descriptive statistics for each treatment group for all time points assessed, including change from baseline for all post-dose assessments. Shift tables from baseline to post-dose assessments may also be generated, as applicable.

8.3.2.4 Hematology and Chemistry

All hematology and chemistry (continuous variables) parameters will be summarized using descriptive statistics for each treatment group for all time points assessed, including change from baseline for all post-dose assessments. Shift tables from baseline to days 4, 22, 56 and 90 will also be generated for each safety laboratory parameter with values of Within Normal Limits (WNL), High, and Low used for the shift categories.

Laboratory values will be compared to normal range of the laboratory and values that fall outside of the normal ranges will be flagged as: H (High) and L (Low) in the data listings.

8.3.2.5 12-lead ECG

Descriptive statistics will be calculated for 12-lead ECG parameters, including change from baseline for each treatment group for all time points assessed. In addition, the overall interpretation of 12-lead ECG results will be classified using frequency counts and percentages for the categories of Normal, Abnormal (NCS) and Abnormal (CS) for each treatment group for all time points assessed, as applicable. Shift tables from baseline to days 1, 4, and 21 may also be generated, as applicable.

8.3.3 Plasma Concentrations of DM199

Plasma concentrations of DM199 will be analyzed using the Safety population. DM199 concentrations will be summarized by treatment group at days 2, 22 and 90, as applicable.

8.3.4 Efficacy Endpoints

Efficacy endpoints will be analyzed using the mITT population.

Efficacy endpoints (continuous variables) will be summarized using descriptive statistics for each treatment group for all time points assessed, including change from baseline for all post-dose assessments (days 22 and 90), as applicable.

In addition, categorical variables, including the total NIH Stroke Scale score indicating stroke severity, will be summarized using frequency counts and percentages, for each treatment group and all time points assessed, as applicable.

Any additional efficacy analyses will be described in the SAP, as applicable.

8.3.5 Pharmacodynamic Endpoints

PD endpoints will be analyzed using the PD population.

PD endpoints will be summarized using descriptive statistics for each treatment group for all time points assessed, including change from baseline for all post-dose assessments, as applicable.

Additional exploratory analysis may be performed as necessary to further assess primary and secondary study endpoints and may include additional sub-groups as needed. The PD data may also be examined in conjunction with the plasma concentration of DM199 data for any correlations, as applicable.

8.3.6 Diagnostic Biomarker Endpoint

Urine concentration of DM199 will be summarized using descriptive statistics for each treatment group for all time points assessed, including change from baseline for all post-dose assessments, as applicable.

9 RISK/BENEFIT ANALYSIS

9.1 POTENTIAL RISKS

Previous pre-clinical and clinical studies have shown DM199 to be generally safe and well-tolerated, with postural hypotension as a drug-related side effect demonstrated at high doses (refer to **1.2 Nonclinical Summary** and **1.3 Clinical Summary**). The effects of the study treatment on human pregnancy and the unborn fetus are unknown.

Refer to the Investigator's Brochure for full investigational product information including a comprehensive listing of known safety information.

9.2 ALTERNATIVE TREATMENTS

Available treatments for acute ischemic stroke include medication treatment with tissue plasminogen activator (tPA) and mechanical thrombectomy. These treatments can only be given within 3-4.5 hours and six hours of stroke onset, respectively.

9.3 POTENTIAL BENEFITS

The impact of DM199 in patients with acute ischemic stroke is unknown. However, it is possible that treatment with DM199 may improve blood flow and other mechanisms that may promote long term recovery in patients recovering from acute ischemic stroke. As with all investigational products, the long-term results of using DM199 are not known at this time.

9.4 RISK MINIMIZATION

DM199 is being evaluated for use as intended in treating acute ischemic stroke in the patient population defined herein. This protocol is specifically designed to manage and minimize risk through careful subject selection, training of Investigators, adherence to the pre-determined timepoints to assess subject clinical status and regular clinical monitoring visits by trained and qualified Sponsor-appointed personnel.

10 RESPONSIBILITIES

10.1 SPONSOR AND CRO RESPONSIBILITIES

The Sponsor of this study is DiaMedica Therapeutics Inc. (Collingwood, Victoria, Australia). DiaMedica has retained Novotech (Sydney, Australia) as the contract research organization responsible for executing the obligations of the Sponsor as allowed by 21 CFR, Part 312.52. A Novotech clinical project team will be developed and trained to carry out the obligations of the Sponsor as required by 21 CFR, Part 312, Subpart D and will adhere to Novotech internal procedures, 21 CFR, parts 11, 54, 56, 312, all other applicable regulations, and ICH E6 Guidance for Good Clinical Practice (GCP).

Novotech is responsible for ensuring the study is conducted in accordance with the clinical study protocol and applicable federal regulations (21 CFR, Part 312, Subpart D). In addition, the CRO is responsible for the following:

- Selecting qualified Investigators and providing Investigators with appropriate information for study conduct
- Ensuring proper monitoring of study progress by monitors qualified by training and experience
- Securing compliance to the protocol and applicable regulations when necessary
- Providing all Investigators with an Investigator's Brochure and ensuring that the necessary regulatory agencies and participating Investigators are promptly informed of significant new adverse effects or risks with respect to the investigational product
- Obtaining financial disclosure information from all participating Investigators prior to study commencement and a commitment from Investigators to promptly update the information if relevant changes occur during the study and for one year following study completion
- Maintaining adequate study records and permitting review of these records in the event of a regulatory inspection

- Ensuring proper oversight of the receipt, shipping, and disposition of all investigational product throughout the study period

10.2 INVESTIGATOR RESPONSIBILITIES

Investigators are responsible for ensuring the investigation is conducted in accordance with the protocol and applicable federal regulations (21 CFR, Part 312, Subpart D). Investigators are also responsible for:

- Obtaining initial IRB/EC review prior to commencing the clinical study as required by 21 CFR, Part 56
- Protecting the rights, safety, and welfare of study subjects
- Obtaining informed consent of study subjects prior to enrollment into the clinical study
- Ensuring investigational product is only provided to qualified study subjects enrolled in the trial and is securely stored in a locked, limited access area
- Record retention as defined in federal regulations 21 CFR, Part 312.62 (a) and (b), including disposition of investigational product and adequate and accurate case histories
- Submitting accurate reports as defined in federal regulations 21 CFR, Part 312.64, including:
  - Progress reports submitted at least annually to the IRB/EC and CRO,
  - Safety reports (refer to **6.5 Expedited Reporting of Serious Adverse Events**),
  - Final report submitted to the IRB/EC and CRO upon completion of the Investigator's participation in the study, and
  - Financial disclosure if any relevant changes occur during the course of the study and for one year following completion of the study (refer to **13.10 Financial Disclosure**)
- Permit access to study records for monitoring and auditing activities (refer to **12. Study Monitoring and Auditing**)
- Ensuring that all persons assisting with the trial are adequately qualified, informed about the protocol and any amendments, the study treatments, and their trial-related duties and functions

11 TRAINING

11.1 SITE AND INVESTIGATOR SELECTION

Novotech will select qualified Investigators and provide them with the necessary information to properly conduct the study (21 CFR, Part 312, Subpart D). During the site selection/qualification process, Novotech personnel will assess the adequacy of the facility, the availability of the Investigator, the potential number of study participants, and the provisions for staff support.

11.2 SITE INITIATION

A site initiation visit will include training of the Investigator and site staff on the protocol, relevant regulations, IRB/EC review and approval, completion and submission of eCRFs, and

record keeping requirements. Once the site initiation visit has been completed and the Novotech designee determines all training and regulatory requirements have been satisfied, communication will be sent to the Investigator approving the site to enroll subjects.

11.3 ONGOING TRAINING

Training may be conducted throughout the course of the clinical study if changes are made to the protocol, for non-compliance and/or for changes in site personnel during the clinical study. Training may include Investigator meetings, site visits, conference calls and/or web-based training sessions.

12 STUDY MONITORING AND AUDITING

Monitoring activities will be conducted according to the protocol, ICH GCP guidelines, 21 CFR, Part 312, and regulatory guidance relevant to this clinical study.

12.1 MONITORING AND SOURCE DOCUMENT VERIFICATION

Periodic monitoring will be completed on site and/or by remote visit from representatives of the CRO who will check the eCRFs for completeness and accuracy and verify them with source documents. Visits are also intended to review site compliance, administrative records, and to confirm that all adverse events have been reported as required by the protocol. In addition to the monitoring visits, frequent communications (letter, telephone, e-mail and fax) by the study monitors will ensure that the investigation is conducted according to the protocol, regulatory requirements, and good clinical practice.

Study close-out will be performed by the study monitor upon closure of the study. The close-out visit will consist of reconciliation of all remaining data inconsistencies, obtaining current status determination for all unresolved adverse events, and review of administrative records. In addition, the monitor will perform final investigational product reconciliation for all products received, used, shipped, or disposed of during the study.

12.2 ON-SITE AUDITS/INSPECTIONS

An external auditor appointed by the Sponsor or the IRB/EC as well as inspectors appointed by domestic and foreign regulatory authorities may request access to source documents, eCRFs, and other study documents for on-site audits or inspections. Direct access to these documents must be guaranteed by the Investigator, who must provide support at all times for these activities. Medical records and other study documents may be copied during audit or inspection provided that all subject identifiers are removed on the copies to ensure confidentiality.

13 STUDY ADMINISTRATION

13.1 TRIAL STEERING COMMITTEE

A Trial Steering Committee consisting of the National Principal Investigator, Sponsor representative(s), clinician advisors to the Sponsor, and selected site Investigator(s). The committee will include a minimum of five and maximum of seven participants. The purpose of the committee will be to provide oversight for the overall direction and strategy of the trial, review the significance of outcomes data, and act as the trial publication committee. Additionally, the committee may act as an advisory panel for questions regarding informed consent, subject enrollment, protocol implementation, study endpoints, data discrepancies, and other issues that may present during the course of the study. Members of the Trial Steering Committee may participate in open sessions of the DSMB to ensure open communication regarding trial progress and potential safety issues.

13.2 REGULATORY AND ETHICAL CONSIDERATIONS

This clinical study will be conducted in accordance with this protocol, 21 CFR, parts 312, 50, 54, and 56, the Declaration of Helsinki, ICH GCP, applicable federal and local regulatory requirements, and the conditions of approval imposed by the reviewing IRB/EC.

13.3 PROTOCOL DEVIATIONS

A protocol deviation is defined as any intentional or unintentional change to, or noncompliance with, the approved protocol procedures or requirements. Deviations may result from the action or inaction of the subject, Investigator, or site staff. All deviations will be tracked and should be reported to the IRB/EC in accordance with their reporting policy. Examples of deviations include, but are not limited to:

- Failure to obtain informed consent from subjects or legally authorized representatives before performing study-specific procedures
- Failure to adhere to study inclusion and exclusion criteria
- Failure to comply with dosing requirements, including administering study treatment outside of the time frame specified in the protocol
- Failure to maintain the Investigator or subject blind
- Use of medications that are specifically prohibited in the protocol
- Missed or out-of-window visits
- Failure to adhere to test requirements, including testing not done, incorrect tests done, or not done within the time frame specified in the protocol
- Incorrect storage of study treatment
- Failure to update the ICF when new risks become known
- Failure to obtain IRB/EC approvals for the protocol and ICF revisions

13.4 COMPLIANCE OVERSITE AND STUDY TERMINATION

The Sponsor and its CRO representatives will review and monitor Investigator compliance and determine if there is a need to secure compliance based on the severity and/or trends in non-compliance to the signed agreement, protocol, applicable regulations, or any conditions of approval imposed by the reviewing IRB/EC or regulatory agency (21 CFR, Part 312.56). Depending on the severity and/or trend in non-compliance, the Investigator may receive a formal warning or retraining through a site visit or conference call.

DiaMedica retains the right to suspend or terminate the participation of a site and/or Investigator for any of, but not limited to, the following reasons:

- Failure to secure informed consent from subjects or legally authorized representatives
- Repeated non-compliance with the protocol, applicable regulations, or requirements set forth by the IRB/EC
- Inability to successfully implement the protocol
- Falsification of data, or any other breech of ethics or scientific principles
- Administrative decision such as low enrollment rates or enrollment targets met

Written notice will be submitted to the Investigator in advance of such suspension or termination. The Sponsor will also send a report outlining the reasons for suspension or termination to the IRB/EC and Regulatory Authorities as required by regulation. A suspended or terminated site may not be reinstated without approval of the reviewing IRB/EC and Regulatory Authorities, as required by regulation.

Investigational product will be immediately returned to the Sponsor or designee, unless this action would jeopardize the rights, safety, or welfare of the subject(s).

The Principal Investigator at any investigational site reserves the right to discontinue the study for safety reasons at any time in collaboration with the Sponsor.

13.5 CONFIDENTIALITY

All data and records generated during this study will be kept confidential in accordance with institutional and IRB/EC policies on subject privacy. The Investigator and other site personnel will not use such data and records for any purpose other than conducting the study.

Subject names will not be provided to the Sponsor. Only the subject number and initials will be recorded in the eCRF. If the subject name appears on any document (e.g., laboratory report), it must be eliminated/redacted on the copy of the document supplied to the Sponsor. Study findings stored on a computer will be kept in accordance with local data protection laws. Subjects will be informed that representatives of the Sponsor, IRB/EC, or regulatory authorities may review their medical records to verify the information collected, and that all personal information made available for inspection will be handled in strictest confidence and in accordance with local data protection laws.

The Investigator will maintain a subject identification list (subject number with the corresponding patient name) to enable records to be identified.

13.6 PROTOCOL AND AMENDMENT APPROVALS

Prior to initiation of the study at the study sites, written IRB/EC approval of the protocol and ICF that are based on applicable regulations and the principles of ICH GCP procedures will be obtained. This approval will be typed on the institutional letterhead and will refer to the study protocol and ICF by title and protocol number and will also include date of each document. A copy of the signed and dated letter of approval will be provided to Novotech prior to study commencement. Any written information and/or advertisements to be used for subject recruitment will be approved by the IRB/EC prior to use. A list of the IRB/EC voting members, their titles or occupations, and their institutional affiliations will be requested before study initiation and maintained throughout the duration of the study.

Protocol amendments will be submitted to regulatory authoritie(s) by the Sponsor or designee if required by applicable regulation (21 CFR, Part 312.30) and approved by each site IRB/EC prior to adoption at that site.

13.7 ONGOING INFORMATION FOR IRB/EC AND HEALTH AUTHORITIES

The Sponsor must submit the following to all Investigators, the IRB/EC, and regulatory agencies:

- Information on serious or unexpected AEs from any investigational site, as soon as possible
- Expedited safety reports, in accordance with local and national regulations
- Periodic reports on the study progress (at least annually)

13.8 RECORD RETENTION

All study documents must be retained by the Investigator for at least 15 years. Beyond this period, the Investigator must obtain approval in writing from the Sponsor before the destruction of any records.

The documents to be retained include:

- Original signed informed consent documents for all subjects
- Patient identification code list, screening log, and enrollment log
- Record of all communications between the Investigator and the IRB/EC, Sponsor/CRO, Medical Monitor, Safety Review Committee, and other Investigators
- List of Sub-Investigators and other appropriately qualified persons to whom the Investigator has delegated trial-related duties, together with their roles in the study and their signatures and dates of participation
- Investigational product accountability records
- Record of any body fluids or tissue samples retained

- All other source documents (subject medical records, hospital records, laboratory records, etc)
- All other documents as listed in Section 8 (Essential Documents for the Conduct of a Clinical Trial) of the ICH Guidance E6: Good Clinical Practice: Consolidated Guidance

The Sponsor must be contacted if the Investigator plans to leave the investigational site to ensure that arrangements for a new Investigator or records transfer are made prior to the Investigator's departure.

13.9 LIABILITY AND INSURANCE

Liability and insurance provisions for this study are given in separate agreements. The Sponsor has taken out an insurance policy covering their civil responsibility.

13.10 FINANCIAL DISCLOSURE

Before the start of the study, the Investigator and each Sub-Investigator will disclose to the Sponsor any proprietary or financial interests he or she may hold in the investigational products or the Sponsor company as outlined in the financial disclosure form provided by the Sponsor. The Investigator and each Sub-Investigator also agree to update this information in case of significant changes during the study or within one year of study completion. The Investigators and each Sub-Investigators also agree that, where required by law or regulation, the Sponsor may submit this financial information to domestic or foreign regulatory authorities in applications for marketing authorizations.

14 INFORMATION DISCLOSURE AND INVENTIONS

14.1 OWNERSHIP

All information provided by DiaMedica and all data and information generated by the sites as part of the study (other than a subject's medical records), are the sole property of DiaMedica. All rights, title, and interests in any inventions, know-how, or other intellectual or industrial property rights which are conceived or reduced to practice by site staff during the course of or as a result of the study are the sole property of DiaMedica. and are hereby assigned to DiaMedica. If a written contract for the conduct of the study which includes ownership provisions inconsistent with this statement is executed between DiaMedica and study sites, the contract provisions with respect to ownership shall prevail.

14.2 CONFIDENTIALITY/USE OF STUDY FINDINGS

All information concerning the investigational product and any matter concerning the operation of the Sponsor, such as clinical indications for the drug, its formula, methods of manufacture and other scientific data relating to it, that have been provided by the Sponsor and are unpublished, are confidential and must remain the sole property of the Sponsor. The Investigator will agree to

use the information only for the purposes of carrying out the study and for no other purpose unless prior written permission from the Sponsor is obtained.

The Sponsor has full ownership of the original eCRFs completed as part of this study.

By signing the clinical study protocol, the Investigator agrees that the results of the study may be used for the purposes of national and international registration, publication, and information for medical and pharmaceutical professionals. The authorities will be notified of the Investigator's name, address, qualifications, and extent of involvement.

The Sponsor will ensure that a clinical study report on the study is prepared.

All materials, documents, and information provided by the Sponsor to the Investigator, and all materials, documents and information prepared or developed in the course of the study to be performed under this protocol, shall be the sole and exclusive property of the Sponsor. Subject to obligations of confidentiality, the Investigator reserves the right to publish only the results of work performed pursuant to this protocol provided, however, that the Investigator provides an authorized representative of the Sponsor with a copy of the proposed publication for review and comment at least 45 days in advance of its submission for publication. If requested, the Investigator will withhold publication an additional 90 days to allow for filing a patent application or taking such other measures the Sponsor deems appropriate to establish and preserve its proprietary rights.

It is agreed that, consistent with scientific standards, publication of the results of the study shall be made only as part of a publication of the results obtained by all sites performing the protocol.

14.3 CONFIDENTIALITY

All information provided by DiaMedica and all data and information generated by the sites as part of the study, (other than a subject's medical records), will be kept confidential by the Investigators and other site staff. The Investigators or other site personnel will not use this information and data for any purpose other than conducting the study. These restrictions do not apply to: (1) information which becomes publicly available through no fault of the Investigator or site staff; (2) information which is necessary to disclose in confidence to an IRB/EC solely for the evaluation of the study; (3) information which is necessary to disclose in order to provide appropriate medical care to a study subject; or (4) study results which may be published as described in the next paragraph. If a written contract for the conduct of the study which includes confidentiality provisions inconsistent with this statement is executed, that contract's confidentiality provisions shall apply rather than this statement.

14.4 PUBLICATION

No publication of the results shall take place without the express consent of DiaMedica. Prior to submitting for any publication, presentation, use for instructional purposes or otherwise disclosing the study results generated by the sites (collectively, a "publication"), the Investigator

shall provide DiaMedica with a copy of the proposed publication and allow DiaMedica a period of at least 30 days (or for abstracts, at least five working days) to review the proposed publication. Proposed publications shall not include DiaMedica's confidential information. At the request of DiaMedica., the submission or other disclosure of a proposed publication will be delayed for a sufficient length of time to allow DiaMedica to seek patent or similar protection of any inventions, know-how or other intellectual or industrial property rights disclosed in the proposed publication. If a written contract for the conduct of the study, which includes publication provisions inconsistent with the statement is executed, that contract's publication provisions shall apply rather than this statement.

15 REFERENCES

Cheng NT, Kim AS. Intravenous thrombolysis for acute ischemic stroke within 3 hours versus between 3 and 4.5 hours of symptom onset. Neurohospitalist. 2015;5(3):101-9.