SUPPLEMENT 1
Protocol

Stroke Hyperglycemia Insulin Network Effort (SHINE) Trial Protocol

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SPONSOR

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Investigator's Agreement

I have read the attached clinical protocol titled Stroke Hyperglycemia Insulin Network Effort (SHINE) dated 10/23/2012 and agree to conduct the protocol as written in this document.

I agree to comply with the Declaration of Helsinki/Tokyo/Venice on Experimentation in Humans as required by the United States Food and Drug Administration regulations; the Code of Federal Regulations Title 21 parts 50, 56, 312, 800, as applicable; the Code of Federal Regulations Title 45 part 46; International Conference on Harmonisation Good Clinical Practice Guidelines; and all other applicable guidelines.

I understand this document contains confidential information of SHINE Executive Committee, the NETT CCC and SDMC and cannot be disclosed to anyone other than members of my staff conducting this trial and members of my Institutional Review Board or Ethical Committee.

I agree to ensure that this information will not be used for any purpose other than the evaluation or conduct of this clinical trial without the prior written permission of the SHINE Executive Committee.

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Signature of Site Principal Investigator Date

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Printed name of Site Principal Investigator

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Signature of Co-Principal Investigator (When applicable) Date

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Printed name of Co-Principal Investigator (When applicable)

ABBREVIATIONS

Abbreviation	Description
ADA	American Diabetes Association
AE	Adverse Event
AHA	American Heart Association
ALIAS	High-Dose Albumin Therapy for Neuroprotection in Acute Ischemic Stroke
ASAP	Acute Stroke Accurate Prediction
ATLANTIS	Alteplase Thrombolysis for Acute Noninterventional Therapy in Ischemic Stroke
BG	baseline glucose
BI	Barthel Index
CCC	Clinical Coordinating Center
CI	confidence interval
CRF	case report form
D/C	discharge
DSMB	Data and Safety Monitoring Board
DVT	deep venous thrombosis
EC	Executive Committee
ED	Emergency Department
FDA	Food and Drug Administration
GIST-UK	Glucose Insulin in Stroke Trial – United Kingdom
GRASP	Glucose Regulation in Acute Stroke Patients
IA	intra-arterial
ICH	intracranial hemorrhage
IND	Investigational New Drug
IRB	Institutional Review Board
IV	intravenous
LAR	Legally Authorized Representative
MSM	Medical Safety Monitor
MOP	Manual of Procedures
NETT	Neurological Emergency Treatment Trials
NIH	National Institutes of Health
NIHSS	NIH Stroke Scale
NINDS	National Institute of Neurological Disorders and Stroke
mRS	modified Rankin Scale
PI	Principal Investigator
PO	by mouth
q	every
RX	treatment
SAP	Statistical Analysis Plan
SAE	Serious Adverse Event
SDMC	Statistical Data Management Center
SHINE	Stroke Hyperglycemia Insulin Network Effort
SQ	subcutaneous

ABBREVIATIONS

Abbreviation	Description
SSQOL	Stroke Specific Quality Of Life
THIS	Treatment of Hyperglycemia in Ischemic Stroke
TOAST	Trial of ORG 10172 in Acute Stroke Treatment
tPA	Tissue Plasminogen Activator

1. SUMMARY

There is an increasing need for improved treatments for stroke patients as stroke is the most common cause of serious long term adult disability and the third most common cause of death in the United States.[1] Hyperglycemia is seen in approximately 40% of acute ischemic stroke patients[2,3] and has been associated with worse clinical outcomes.[4,5] Intravenous (IV) insulin therapy with tight glucose control has been found to improve clinical outcomes in some non-stroke acute illness trials.[6,7] Current stroke guidelines emphasize the need for definitive clinical trials to determine best practice for managing hyperglycemia in acute stroke patients.[8] A clear determination of the risk and benefit of glucose control with IV insulin would have a dramatic impact on acute ischemic stroke patient therapy.

This Phase III multicenter, randomized, controlled trial will determine the efficacy and provide further safety data on glycemic control in stroke patients. The hyperglycemic acute ischemic stroke patients that meet all eligibility criteria will receive up to 72 hours of hyperglycemia control with IV insulin therapy or control therapy with subcutaneous (SQ) insulin. Treatment will be given within 12 hours of symptom onset and is recommended, but not required, to begin within 3 hours of arrival to the emergency department (ED). The primary efficacy outcome to be assessed at 90 days will be the severity adjusted difference in favorable outcome between the groups. Favorable outcome will be defined by a previously described baseline severity adjusted dichotomized modified Rankin scale (mRS).[9-11] Outcome success will depend on the severity of the initial stroke (per NIH Stroke Scale Score (NIHSS)). The primary safety outcome will be the hypoglycemic event rate. Secondary outcomes will assess additional neurological and functional status using stroke severity, functional and quality scales[12-14] as well as glucose control success and adherence to the protocol dosing recommendations of the computerized decision support tool. This trial launches a highly collaborative model for stroke research providing a foundation for maximally generalizable results based on performance at academic, community, urban, rural, large and small hospitals throughout North America to produce a highly representative national population sample. A validated computer decision support tool will guide delivery of IV insulin therapy. A baseline severity-adjusted dichotomized outcome analysis (responder analysis)[9] will adjust for variability of individual patient characteristics to allow detection of the true clinically relevant treatment effect. In this setting **an absolute 7% treatment effect is recognized as a threshold at or above which a profound effect on a large stroke population would be realized**.

2. OBJECTIVES

2.1 Specific Aim 1

To determine the efficacy of tight glucose control to a target range of 80-130 mg/dL with IV insulin infusion in hyperglycemic acute ischemic stroke patients within 12 hours of symptom onset as measured by mRS at 90 days after stroke.

- Hypothesis 1: Tight glucose control (target 80-130 mg/dL) with IV insulin infusion therapy using a validated computerized decision support tool, will increase the severity adjusted 90 day favorable outcome on the mRS by an absolute 7% or more, as compared to the control group.

2.2 Specific Aim 2

To determine the safety of tight glucose control with IV insulin infusion in hyperglycemic acute ischemic stroke patients treated for up to 72 hrs.

- Hypothesis 1: Tight glucose control with IV insulin infusion therapy using a decision support tool is safe as determined by a severe hypoglycemia (<40 mg/dL) rate that does not exceed that of the control group by more than 4%.

3. BACKGROUND AND RATIONALE

3.1 Background

Ischemic Stroke represents a large burden to society with only a single proven acute treatment. IV Thrombolytic therapy has proven applicable to only a small minority of stroke patients who present within a narrow time window. Even for those patients receiving this therapy, the chances of recovery to normal or near normal are only increased by approximately 30%. Sorely needed are additional new stroke treatments applicable to a larger universe of early acute stroke patients which are safe and efficacious when delivered over an expanded time window. Hyperglycemia, seen in large numbers of acute stroke patients and well associated with poorer clinical outcomes, provides a compelling target for intervention.

3.2 Significance

Stroke remains the third leading cause of death and leading cause of adult disability in the U.S. The total cost of stroke for 2009 is estimated at nearly $69 billion.[1] Stroke occurs in nearly 800,000 people annually in the United States[1] with approximately 85% (637,500) of them being ischemic and approximately 40% (250,000) of the ischemic strokes being hyperglycemic (≥130 mg/dL) at presentation to the hospital.[2,3] An efficacious and effective treatment for hyperglycemia in this population would have an enormous impact. Preliminary data have demonstrated the safety and feasibility of insulin infusion therapy in acute ischemic stroke patients, but efficacy remains unknown.[15-21] Hyperglycemia is associated with worse outcomes and yet hypoglycemia is bad for ischemic brain. Treatment for hyperglycemia with a very low hypoglycemia rate is highly likely to be beneficial. The stroke community has struggled with uncertainty regarding how hyperglycemic stroke patients should be managed and the most recent American Heart Association (AHA) guidelines suggest that the management uncertainty in acute stroke patients will require clarification by clinical trials.[8] Health care providers are currently making clinical decisions regarding hyperglycemic management without adequate data. The guidelines classify the current evidence as C (consensus of experts) only. The information gained from this efficacy trial will guide clinical practice and provide answers

regarding the risk/benefit ratio of glucose control using insulin infusion therapy to improve stroke outcomes. Even a small beneficial effect of an absolute 7% (roughly half of IV tPA) is likely to gain the attention of physicians who care for stroke patients worldwide and likely to change current practice for over 250,000 patients per year as great variability now exists in this setting of uncertainty. Evidence of unacceptable risk/benefit ratio would guide clinicians to avoid this therapy and redirect resources and efforts toward other promising acute stroke therapies. The additional information from the SHINE trial will provide knowledge that will advance the field and may improve clinical outcomes in stroke patients.

Table 1. Studies assessing clinical outcomes associated with <u>admission</u> or <u>in-hospital</u> hyperglycemia in ischemic stroke adjusted for confounding factors.

Study, 1st author, year	**Patien**	**Main Result**
Mortality with <u>admission</u> hyperglycemia		
Moulin, 1997[22]	1776	Increased 30 day mortality, RR 1.007 (1.004, 1.010)
Williams, 2002[3]	634	Increased 30 day mortality if glucose ≥130 mg/dL, HR 1.87 (1.05,3.32), p=0.018
Gentile et al, 2006[2]	960	Increased mortality if glucose ≥130mg/dL, p<0.004
<u>Reduced favorable clinical outcome at 3 months with admission hyperglycemia</u>		
Weir, 1997[23]	645	p<0.001. Only patient w/o diabetes analyzed
TOAST, Bruno, 1999[24]	1259	p=0.03. All study patients (Rx and placebo)
Demchuk, 2001[25]	616	p= 0.03. All patients treated with tPA
ATLANTIS, Bruno, 2002[26]	755	p<0.001. All study patients, (tPA and placebo)
NINDS tPA, Bruno, 2002[26]	624	p=0.02. All study patients (tPA and placebo)
CASES, Poppe, 2009[27]	1098	RR=0.7 (0.5,0.9) if glucose >144mg/dL (tPA patients)
<u>Worse clinical outcomes with in-hospital hyperglycemia</u>		
Gentile et al, 2006[2]	960	Increased mortality if glucose ≥130 mg/dL, p<0.001
ECAS-II, Yong, 2008[28]	587	Reduced favorable outcome if glucose >140 mg/dL, OR=0.36 (0.13,0.71)
Fuentes, 2009[29]	476	Increased poor outcome if glucose ≥155 mg/dL, p=0.002
Dziedzic, 2009[30]	689	Increased 90 day mortality, HR 1.10 (1.03,1.18), p<0.01

TOAST = Trial of ORG 10172 in Acute Stroke Treatment;
ATLANTIS = the acute stroke rt-PA 3-5 hours after onset of symptoms treatment trial;
NINDS rt-PA = the NINDS rt-PA Acute Stroke Trial;
CASES = Canadian Alteplase Stroke Effectiveness Study

Of importance is the finding that glucose concentrations after admission are also associated with worse clinical outcomes. These data suggest that clinical outcomes may be impacted by normalization of blood glucose in the first few days after stroke.

Table 2. Association between acute hyperglycemia and infarct volume on MRI			
1st author, yr	**Number of**	**Definition of hyperglycemi**	**Outcome associ**
Parsons, M 2002[31]	40	>144 mg/dL	Larger infarcts, less penumbral salvage, higher brain lactate
Els T, 2002[32]	31	>178 mg/dL	Greater lesion expansion
Baird TA, 2003[33]	20	>126 mg/dL	Greater lesion expansion
Ribo M, 2007[34]	47	>140 mg/dL	Greater lesion expansion

In summary, the majority of data from observational studies show an independent association between both admission and in-hospital (first several days) hyperglycemia with worse clinical and imaging outcomes in acute ischemic stroke patients.

3.3 Hyperglycemia Correction Trials: Acute Ischemic Stroke

The Glucose Insulin in Stroke Trial – United Kingdom (GIST-UK)[20] was intended to be a definitive efficacy trial to address aggressive hyperglycemia correction in acute stroke patients (ischemic and hemorrhagic). Unfortunately,
this multicenter, controlled trial did not provide adequate efficacy data as the trial was stopped early for financial reasons with only 40% enrollment, so was underpowered, and because both treatment groups achieved glucose concentrations in the treatment target range (72-126 mg/dL) that was intended only for the insulin infusion group.[20] No difference in outcomes between the groups was detected. Important information however can be gleaned from the GIST-UK trial that informs future trials.

The GIST-UK trial excluded insulin treated patients with diabetes. Only 17% of the patients had diabetes (all non-insulin treated) while 83% did not have diabetes. Consequently, despite only saline infusion in the control group, the mean glucose during protocol treatment was in the intervention target range for both groups (Figure1). Though the treatment was safe, these data demonstrate that on average, patients without diabetes normalize their glucose concentrations spontaneously without need for glucose control intervention, as has been reported elsewhere.[35] As animal data and observational clinical studies suggest it is likely that glucose concentration and not the presence of insulin is related to improved outcomes, the comparison of two groups in the same target range in the GIST-UK trial would not be expected to demonstrate a difference in clinical outcome. Despite the limitations of the GIST–UK trial it has informed the SHINE trial to include patients with diabetes to allow for the two treatment groups to have separation of glucose concentrations in two different target ranges.

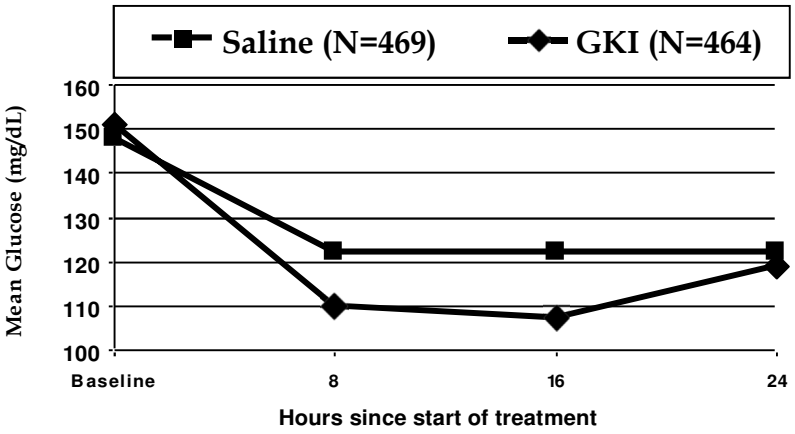

Figure 1. GIST-UK trial[20] **glucose concentrations** (data converted from mm/liter to mg/dL). Graph demonstrates that both groups are in the intervention target range

In addition to our middle phase (pilot) trials, THIS and GRASP, described in the preliminary data section below, there are two additional published middle phase trials. Walters, et al[19] studied the safety and feasibility of hyperglycemia correction in acute ischemic stroke in 25 patients with hyperglycemia (>110 mg/dL) randomized within 24-hours after stroke onset to IV insulin therapy or IV saline (control) with continuation of previous oral antidiabetic drugs. Of these patients 52% had diabetes mellitus. The intervention lasted 48 hours and target glucose was 90-144 mg/dL in the insulin infusion group. The mean blood glucose achieved was 122 mg/dL in the insulin infusion group and 145 mg/dL in the control group. There was one episode of hypoglycemia with autonomic symptoms only in the insulin infusion group (glucose 72 mg/dL). Importantly, they also demonstrated that glucose concentrations normalize with minimum intervention in patients without diabetes. The investigators concluded that glycemic control was safe and feasible. In another pilot trial, Kreisel et al[21] randomized 40 patients with acute ischemic stroke within 24 hours regardless of admission blood glucose to standard subcutaneous or IV insulin treatment. The target glucose range in the insulin infusion group was 80-110 mg/dL. Of these patients 33% had diabetes mellitus. In patients without diabetes the glucose concentrations were normal throughout the 5 day protocol period in both treatment groups. In patients with diabetes, the glucose concentrations were higher in the standard treatment group than in the IV insulin treatment group (180-205 mg/dL in the standard group and 120-150 mg/dL in the IV insulin group on days 1- 3). Hypoglycemia (<60 mg/dL) developed in 7 (35%) patients, with autonomic symptoms in 3 (15%), and none had neurological hypoglycemic symptoms.

3.4 THIS and GRASP Preliminary Data

The investigators have substantial preliminary data from two NINDS funded pilot trials (THIS[16] and GRASP[17]) as well as data from a recent NETT stroke trial that inform the design of SHINE.

THIS Trial Description and Results

The Treatment of Hyperglycemia in Ischemic Stroke (THIS) trial was an NINDS funded safety and feasibility multicenter pilot trial of aggressive hyperglycemia

reduction in acute ischemic stroke.[16] Patients (N=46) were randomized within 12 hours of stroke onset to continuous IV insulin infusion (N=31) (target 80-130 mg/dL) or sliding scale SQ insulin injections up to four times daily if needed (control N=15). The baseline glucose threshold for inclusion was 150 mg/dL and 91% were diabetic.

The main findings in THIS were safety and feasibility. Elevated glucose levels were lowered into target range with mean glucose nearly 60 mg/dL (31%) lower in the IV insulin group than the SQ insulin group (133 vs. 190 mg/dL) within four hours of starting therapy.

THIS Safety Results

There were no serious adverse events related to the IV insulin intervention. Overall, episodic hypoglycemia (<60 mg/dL) occurred in 11 patients (11/31, 35%) in the IV insulin group. Seven of these hypoglycemic patients were asymptomatic. There was only one patient who had transient neurologic symptomatic hypoglycemia. No patient had severe (<40 mg/dL) or prolonged hypoglycemia. Ten patients (22%) were treated with standard IV tPA within three hours of stroke onset and no treatment related adverse events were seen in this subgroup. Glucose concentrations were consistently and significantly lower throughout the trial in the IV insulin group. The adjusted mean glucose difference between the groups during treatment was 66 mg/dL. Non-diabetic patients had glucose levels return to normal without aggressive treatment.

THIS Clinical Outcomes

There were no statistically significant differences in clinical outcomes between the two treatment groups. The primary exploratory analysis compared mRS ≤2 at 3 months. Favorable outcome was more common in the aggressive treatment group, by an absolute 5%. Secondary favorable clinical outcomes were also more favorable in the IV insulin group. The largest difference was in the NIH Stroke Scale ≤2, with p=0.09 in favor of the IV insulin after adjusting for baseline inequalities. No patient was lost to follow up.

GRASP Trial Description and Results

The GRASP trial (N=74) was a multicenter randomized safety, feasibility, “dose finding” trial that included 3 treatment groups (usual, loose and tight control).[17] Patients were enrolled within 24 hours of stroke and treated for up to 5 days. Patients with diabetes in the trial had a mean glucose of 185 mg/dL and patients without diabetes had a mean glucose of 149 mg/dL at randomization. These data support a lesser need for insulin therapy in stroke patients without diabetes.

GRASP Protocol Insulin Treatments

1. Insulin Infusion Protocol (eProtocol-insulin)

eProtocol-insulin is a computerized decision support tool used in the GRASP trial. Both loose and tight control groups received continuous insulin infusion based on eProtocol-insulin. Overall, there was a 97% adherence to eProtocol

recommendations suggesting that nurses agreed with the computer recommendation and followed the study protocol.

2. Control Group

The control group was a community control with the intent to observe standard care and to inform the control group in future trials. A total of 88% of control patients were treated with SQ insulin.

GRASP Safety

Total hypoglycemia rates (<55 mg/dL) were 4% in the control group, 4% in the loose control group, and 30% in the tight control group. One patient in the loose control group had transient symptomatic hypoglycemia with a glucose of 50 and 52 mg/dL that was rapidly corrected. A total of 26 patients (36% of enrolled subjects) received standard IV thrombolysis within 3 hours. The Data Safety Monitoring Board (DSMB) and the safety monitor had no safety concerns in this group. There were 10 (14%) deaths in this trial. No patient had severe or prolonged hypoglycemia.

GRASP Glucose Concentrations and Feasibility

The median glucose during treatment in the control group was 151 mg/dL, in the loose group was 151 mg/dL, and in the tight group was 111 mg/dL. Target glucose levels were achieved. They also demonstrate that IV insulin is not necessary for the loose target as the same glucose control can be achieved with SQ insulin.

GRASP Recruitment/Enrollment

Recruitment and enrollment were highly successful with completion of enrollment of 74 subjects within 18 months and ahead of schedule. The recruitment specialist was critically important to the recruitment success and because of her expertise and commitment she will oversee recruitment in the SHINE trial.

GRASP/THIS Target Glucose Concentration ("dose") Selection

Both GRASP IV insulin doses are safe and feasible, but the tight control approach is appropriate to study in the phase III trial. GRASP data, combined with THIS trial data (target 80-130 mg/dL), the AHA guidelines (treat glucose over ~180 mg/dL), the ADA guidelines (treat over180mg/dL) and the literature reviewed above support an intervention group target glucose of 80–130 mg/dL and a control group treated with SQ insulin for glucose >180 mg/dL. Separation will be achieved as diabetics will be the study population and our preliminary data demonstrate we can adequately separate levels.

GRASP/THIS Time Window

GRASP had a 24 hour window for enrollment, but 58% of patients were enrolled <12 hours from stroke onset and 45% <9 hours. These data with the THIS trial demonstrate that patients can be enrolled within 12 hours to an acute IV insulin trial. A 12 hour window will allow tPA therapy for eligible patients, allow "wake

up stroke" enrollment and is supported by the literature. Data suggest that both inflammation and excitotoxicity likely play a major role in the injury pathophysiology that tight glucose control is interrupting.[36] TNF alpha may be a major component of the injury pathway and is expressed by 4 hours but doesn't peak until 12 hours.[37] A 12 hour window therefore may have greater ability to capture patients that will benefit from this therapy. The rapid sequence of evaluation and study enrollment for acute stroke patients will assure that patients will be enrolled quickly and many will likely be early in the time window (as shown in GRASP). This rapid enrollment will be maximized in our collaboration with the NETT and will allow a secondary analysis of time to treatment as we are likely to have a broad distribution of enrollment times.

GRASP/THIS Treatment Period

The GRASP trial treated patients for 5 days or until discharge with the intent of establishing the time period for inpatient therapy. The GRASP data clearly demonstrate that 5 days was not feasible and that 4 days was too long for most patients due to current acute stroke length of stay. Three days was the longest feasible treatment window and was successful in both trials. Additionally, human data suggest better outcomes in patients with glucose control for first 3 days compared to those with hyperglycemia.[38] Pathophysiologic mechanisms that may be involved include protein synthesis dependent protection mechanisms that continue for up to 3 days.[39] Additionally, inflammatory mechanisms (suspected to be involved) are known to continue for several days. Animal model data suggest that 3 days of anti-inflammatory treatment can reduce infarct volume.[40] These mechanistic data plus our pilot feasibility data strongly suggest that a 3 day treatment period is ideal. Mild stroke patients will be discharged as clinically indicated which may be <3 days.

GRASP/THIS Meal Insulin

The GRASP and THIS trials used a simple algorithm for estimating carbohydrate consumption and providing meal insulin. One unit/15 grams carbohydrate consumed will be used in SHINE.

GRASP Exploratory Efficacy Outcomes (N=73 patients with 3 month outcomes)

None of the efficacy outcome relationships reached statistical significance, but have been used to estimate the control group outcome rates and predict the population most likely to benefit from treatment. The primary exploratory analysis analyzed modified Rankin score ≤1 at 3 months and the tight group had the most favorable outcomes. Three months outcomes were captured on 99% of patients.

Responder Analysis

Based on the well-known statistical principle of loss of information with the use of dichotomized outcomes and the recent literature suggesting that more innovative and powerful analyses may be necessary for "neuroprotection" trials,[41-

[44] we chose to apply the "responder analysis" (baseline adjusted severity) also referred to as "stratified dichotomy analysis" to the SHINE trial. This analysis has been described previously[9] and has confirmed the benefit of tPA in both part 1 and part 2 of the NINDS tPA trials, demonstrating increased power to detect the treatment effect.[10]

The SHINE trial defines a dichotomized mRS score as "favorable" versus "not favorable" based on the baseline NIHSS at randomization as defined previously[9, 10]

Table 3. Stroke Severity Adjusted Stratified Dichotomy[9] analysis.

Trial (NIHSS 3-22 for all)	**Favorable clinical outcome on mRS**	
	Control (95% CI)	**IV Insulin (95% CI)**
GRASP (BG ≥150)	2/11=18% (3,52)	6/15 = 40% (8,68)
THIS	3/15 = 20% (5,37)	4/31 = 13% (4,30)
NINDS tPA (BG≥150)	18/72= 25% (15,37)	N/A
ASAP	55/211= 26% (20,32)	N/A
BG ≥150=baseline glucose ≥150 mg/dL; ASAP = NINDS Acute Stroke Accurate Prediction trial[45]		

Summary Table 3: 4 data sets are analyzed using responders analysis. Control population estimates suggest ~20-25% baseline favorable outcome in a population similar to the SHINE trial population.

Selection of Control and Treatment Group Rates

To determine the appropriate control rate, we assessed the responder analysis from four relevant trials (Table 3). Standard previously published definitions of favorable outcome are used in this trial.[9] Additionally, requested control rate information on a subset similar to the SHINE study population from the Executive Committee of a contemporary stroke trial (ALIAS part 1) has been used in the development of the SHINE protocol. The control population estimates in the 4 trials suggest a reasonable favorable outcome rate of ~20-25% in an acute stroke population similar to that to be enrolled in SHINE. The expected control rate is not anticipated to be an underestimate based on preliminary data. Based on these analyses of 4 recent data sets and 1 old data set (NINDS tPA), we are confident that they support our expected control population rate of favorable outcome (25%). These pilot trials are not intended to be underpowered efficacy trials and therefore do not inform the expected treatment effect. The STAIR group has suggested that an absolute treatment effect of 2-8% is appropriate for neuroprotection trials.[46] A treatment effect rate of 4-6% was felt to be too small an absolute benefit for insulin infusion therapy as it is labor-intensive even if very safe. An absolute treatment effect of 7-8% will change clinical practice and therefore we used an effect size of 7% for our sample size estimation.

Decision Support Tool

The decision support tool, GlucoStabilizer, is an FDA cleared device that has been utilized in over 50,000 patients. The tool has demonstrated extremely low rates of severe hypoglycemia (<40 mg/dL). The severe hypoglycemia rate during use is 1.67% of patients and 0.07% of total glucose readings.

Decision support tools have been used in previous studies, and the insulin infusion patients in the GRASP Trial used eProtocol-insulin, a similar decision support tool. Adherence to the recommendations using decision support tool devices has been reported to be 91-98%,[47, 48] and it was 97% in the GRASP trial using eProtocol[17].

Summary Preliminary Results

The GRASP and THIS trials have shown that insulin infusion therapy for tight glucose control is safe and is feasible in hyperglycemic acute ischemic stroke patients. Additionally, these trials have identified the appropriate glucose target, timing, treatment period, patient population for enrollment, expected loss to follow up rate, expected recruitment rate, and expected control group outcome rates. These pilot data ideally inform the design of the SHINE Trial.

3.5 Rationale

The goal in the treatment group is glucose levels within the target range of 80-130 mg/dL. This requires that the target range be rapidly established and maintained with minimal variability for up to 72 hours. IV insulin infusion due to its rapid onset and offset of action and its superior degree of frequent titratability provides the ideal intervention to achieve and tightly maintain the target range goal across the study period. The use of computerized decision support tools is now part of standard care.

4. STUDY PLAN

4.1 Study Design

SHINE is a multicenter, randomized, controlled clinical trial of approximately 1400 patients to be conducted at 17 Neurological Emergencies Treatment Trials (NETT) hubs and their spoke hospitals as well as approximately 10 non-NETT sites.

4.2 Study Population

The study population will include hyperglycemic acute ischemic stroke patients of either gender who are 18 years of age or older. They will have a history of type 2 diabetes and hyperglycemia or in the absence of diabetes, must have a baseline glucose levels ≥150 mg/dL. To be eligible, treatment must begin within 12 hours of stroke symptom onset and is recommended, but not required, to begin within 3 hours of arrival to the Emergency Department. Participation is limited to those with baseline NIHSS scores 3-22.

4.3 Study Therapy (insulin versus saline)

Insulin is indicated for the treatment of hyperglycemic conditions and the maintenance of euglycemia in the setting of diabetes mellitus. It is also known to be effective in normalizing blood glucose when hyperglycemia results from other conditions. It is frequently used for both the inpatient and outpatient management of patients with diabetes. Insulin given by IV or subcutaneous (SQ) route stimulates carbohydrate metabolism and facilitates transfer of glucose into cardiac muscle, skeletal muscle and adipose tissue, and glucose is converted to glycogen. In addition, lipogenesis is stimulated with inhibition of lipolysis from adipose cells; protein synthesis is stimulated. An Investigational New Drug (IND) approval is not required for this protocol.

Major adverse reactions
Common – Skin site reactions (SQ), Lypodystrophy, Mild hypoglycemia Serious
– Moderate to Severe Hypoglycemia

The SHINE trial will use a validated computerized electronic decision support tool for the IV insulin group only. IV insulin or saline infusions will be adjusted by the nurses to maintain the assigned glucose target. The nurses will consider the recommendation made by the computer for the IV insulin group and will follow the protocol for the saline adjustments.

4.4 Study Decision Support Tool for the Intervention Group

The decision support system, GlucoStabilizer, has been developed by Medical Automation Systems an Alere Company and cleared for use by the FDA. The system provides an alert to the nurse for the next required glucose check. Other decision support tool rules moderate the insulin dose recommendation depending upon previous glucose readings or steep drops in glucose values. Nurses will have the option of accepting or declining the recommended insulin rates. Nurses are encouraged to use clinical judgment and reject any recommendation that seems inappropriate but will be required to stop the insulin drip when notified to do so by the decision support tool.

For blood glucose <80 mg/dL, the decision support tool will instruct the treating team to discontinue the insulin infusion, and investigators will be required to stop the insulin infusion at that time. Sites will be monitored for rates of adherence to the decision support tool recommendation for those patients receiving IV insulin infusion. All glucose measurements, insulin dosing, times of glucose checks and acceptance of recommendations are captured in the electronic system.

4.5 Study Sites

Study sites will include The Neurological Emergency Treatment Trials (NETT) sites and non NETT sites. Details of study sites are listed in the Manual of Procedures (MOP).

4.6 Estimated Study and Enrollment Duration

The project period will begin in late 2011. Enrollment is expected to begin in 2012. Enrollment is anticipated to take 3.5 – 4 years and follow up is 3 months. Final data analysis and close out activities will occur after completion of enrollment and patient follow up.

5. ELIGIBILITY CRITERIA

5.1 Inclusion Criteria

(1) Age 18 years or older

(2) Clinical diagnosis of ischemic stroke defined as acute neurological deficit occurring in one or more cerebral vascular territories. Neuroimaging must be done to exclude intracranial hemorrhage (ICH).

(3) Protocol treatment must begin within 12 hours after stroke symptom onset and is recommended, but not required, to begin within 3 hours after hospital arrival. If time of symptom onset is unclear or patient is awakening with stroke symptoms, the time of onset will be the time the patient was last known to be normal.

(4) Known history of type 2 diabetes mellitus and glucose >110 mg/dL **OR** admission glucose ≥150 mg/dL in those w/o known diabetes mellitus

(5) Baseline NIHSS score of 3-22

(6) Pre-stroke modified Rankin Scale score = 0 for patients with an NIHSS score of 3-7. Pre-stroke modified Rankin Scale score = 0 or 1 for patients with an NIHSS score of 8-22.

(7) Able to provide a valid informed consent to be in the study (self or their authorized legally accepted representative). The approved consent form must be signed and dated in accordance with federal and institutional guidelines.

5.2 Exclusion Criteria

(1) Known history of type1 diabetes mellitus

(2) Substantial pre-existing neurological or psychiatric illness that would confound the neurological assessment or other outcome assessment

(3) Having received experimental therapy for the enrollment stroke. IV tPA (up

to 4.5 hrs) or IA tPA are allowed as are IA therapies including use of

FDA cleared devices. Non FDA cleared devices are considered experimental and are excluded.

(4) Known to be pregnant or breast-feeding at the time of study entry

(5) Other serious conditions that make the patient unlikely to survive 90 days

(6) Inability to follow the protocol or return for the 90 day follow up

(7) Renal dialysis (including hemo or peritoneal dialysis)

Justification for Eligibility Criteria

Only ≥18 year old ischemic stroke subjects will be included since ischemic strokes in the pediatric population are substantially different from adult strokes. The population under age 18 is excluded to avoid confounding the results.

The **12-hour eligibility requirement** was chosen as this is almost universally before the development of maximum edema in acute ischemic stroke patients, but is a wide enough time window to be inclusive of most patients allowing generalizable results and is supported by the preliminary data. Treatment within 3 hours of arrival to the Emergency Department will assure the avoidance of treatment delays in hopes of maximizing treatment effect as suggested by much of the animal and human data in acute ischemic stroke. This will also allow the patients to be treated with standard IV tPA as per published eligibility criteria and then enrolled in the trial. An enrollment **blood glucose >110 mg/dL in patients with type 2 diabetes or ≥150 mg/dL in patients without diabetes** was based on our preliminary data and other data suggesting this group is most likely to benefit.
As demonstrated by GIST-UK[20], THIS[16], GRASP[17], Walters[19] and Kriesel[21] hyperglycemia frequently resolves spontaneously in most patients without diabetes. Thus, patients without diabetes mellitus or admission hyperglycemia will be excluded. Both THIS[16] and GRASP[17] trials and an observational study[45] demonstrated that most patients enrolled with hyperglycemia ≥150 mg/dL remained hyperglycemic during hospitalization unless they received intravenous insulin. The vast majority of patients with admission glucose ≥150 mg/dL ha undiagnosed diabetes or impaired glucose metabolism (insulin resistance) as has been reported,[49-51] thus making them good subjects for this trial. Patients with an NIHSS score of 3-7 will be required to have a **pre-stroke mRS score** of 0 to be eligible. This is intended to allow patients to reach the success criteria defined by the stratified dichotomy outcome. Patients with an NIHSS of 8-22 will be eligible if they have a pre-stroke mRS score of 0 or 1. Patients with **type I diabetes** mellitus are excluded for safety reasons. Usual care for type I diabetes patients during acute illness usually includes intravenous insulin infusion accompanied by dextrose otherwise these patients would be at risk of diabetic ketoacidosis. Since withholding standard care is unacceptable, these patients are excluded. Patients

with type I diabetes will be identified based on medical history. Study definitions of type 1 and type 2 diabetes are provided in the MOP.

Patients with a **neurological or psychiatric illness** likely to confound the final outcome assessment will be excluded since their baseline deficits and outcomes cannot be accurately obtained. Any patient deemed by the enrolling physician to have a condition that confounds the enrollment neurological exam will be excluded. Patients receiving **experimental stroke therapies** will be excluded due to uncertain effects of such therapies on outcomes. Standard care IV tPA or IA
tPA according to the AHA/ASA guidelines will be allowed.[8] The pilot trials demonstrated safety in the population treated with IV tPA. The increased risk of symptomatic hemorrhagic transformation of infarcts observed in IV tPA treated stroke patients with hyperglycemia[25, 26, 52] may be reduced with glucose control. Intra-arterial (IA) treatments that are standard care, including the use of FDA cleared devices, will be allowed. FDA cleared devices must be employed according to their Instructions for Use. Non FDA cleared devices or other experimental interventions will not be allowed. No clear data are available on the risk/benefit ratio of these interventions and they could confound the results. **Pregnant women** will be excluded since the standard care for this population often includes IV insulin treatment for hyperglycemia. Patients **unlikely to return at 90 days** will be excluded since the primary efficacy outcome is measured at that time. **Renal dialysis** patients will be excluded including those requiring hemodialysis and peritoneal dialysis due to inability to accurately follow glucose levels and variability in insulin requirements that would put patients at risk.

5.3 Prohibited Therapy During Study Period

No other diabetes treatment medication besides the assigned protocol treatment will be allowed during the treatment period because such additional medications would confound the study. All oral agents that are used for the treatment of hyperglycemia are unauthorized. The use of non FDA cleared devices for IA therapy is not allowed. Patients taking PO diets must eat protocol specified diets as defined in the MOP.

6. SUBJECT RECRUITMENT

6.1 Methods

The methods used for recruitment of subjects in the study will be devoid of any procedures that may be construed as coercive. The recruitment process will not involve any restrictions on sociodemographic factors including gender or ethnic characteristics of the patient population. However, the composition of the study population will depend on patient sources available to the enrolling sites.

Patients will be recruited by members of the research teams at enrolling sites. Stroke teams and Emergency Medicine teams involved in the immediate evaluation and management of acute stroke patients will be trained to recognize

potentially eligible candidates and to rapidly refer them for formal screening by appropriate study team personal. When a patient is found eligible, they or their Legally Authorize Representative (LAR) as appropriate to the situation will be approached for discussion of the trial and informed consent.

Recruitment and enrollment will usually occur at the acute portal of entry to the enrolling site. Most often this will be in the Emergency Department, but this could also occur in a hospital inpatient unit directly receiving an acute stroke patient in transfer from another facility as long as arrival is within the specified enrollment window.

6.2 Screen Failure Logs

Screen failure logs at each site must be entered into the study database listing all patients with acute ischemic stroke who have presented within 12 hours of onset and are hyperglycemic but **not** randomized into the SHINE Trial. Documentation will include reasons for non-enrollment. Screening data will be reviewed to assure that full efforts are being made with respect to recruitment and enrollment and to identify any patterns with regard to ineligibility or reasons for non- enrollment.

7. SUBJECT ENROLLMENT

7.1 Eligibility Assessment

All patients who present within 12 hours of onset of an acute ischemic stroke will be screened for eligibility for entry into the study. Rapid referral to the study team should be made immediately upon recognition of potentially eligible candidates. Hyperglycemia as defined in the eligibility criteria will be a major trigger for consideration for enrollment.

As in all trials, the goal is to achieve a high level of compliance with protocol requirements by assuring during the eligibility assessment that the potential subject or LAR is fully informed and agrees to the protocol requirements. In addition, patients with a strong likelihood of non-adherence as described in the inclusion and exclusion criteria should not knowingly be randomized. Careful assessment of the patient's understanding of the trial is required prior to enrollment.

7.2 Presentation of Informed Consent

Consent will be obtained by either the site Principal Investigator or by a member of the study team as described in the MOP. The consent should be the IRB- approved version corresponding to the version of the protocol approved when the screening was initiated. Informed consent is to be obtained from the patient or patient's LAR. For the SHINE study, there is no activity required in the screening process that is not typically included within the reasonable scope of standard care evaluation for acute stroke patients; therefore, unless called for by local regulatory

request, patients would be approached for consent only after the clinical screening process had established eligibility.

7.3 Randomization

7.3.1 Central Randomization Procedure

A web-based central randomization system developed by the NETT Statistical Data Management Center (SDMC) and installed on the WebDCU™ SHINE study website.

Subjects will be assigned to one of the treatment groups according to the randomization scheme developed at the SDMC. Randomization must be done centrally using the WebDCU™ for all patients entered in the trial.
In the event that the site cannot access the randomization module of WebDCU™, emergency randomization procedures as defined in the study MOP should be followed.

8. STUDY PROCEDURE

8.1 Baseline Assessments

8.1.1 Point of Care Finger Stick Blood glucose level

Accu-Chek Comfort Curve Test Strip System is a glucose dehydrogenase based system of quantitatively measuring the concentration of glucose in whole blood. A minimum blood sample of 4µL is the required volume on the strip. The reportable range of glucose concentration is 10 – 600 mg/dL. This is the SHINE study preferred test strip. Strips must be stored at < 90◦ F or 32◦ C. Strips should be used at temperatures between 57◦ - 104◦ F and less than 85% humidity. Hematocrit must be between 20-55% for glucose measurements >200mg/dL. Avoid using test strips during xylose absorption testing, in patients receiving maltose infusion, at altitudes above 10,150 feet, in situations of decreased peripheral blood flow to the fingers, galactose level >10 mg/dL, maltose >16 mg/dL, bilirubin (unconjugated) >20 mg/dL, lipemia >5,000 mg/dL, acetaminophen levels >8 mg/dL, uric acid >10 mg/dL.

8.1.2 Concomitant medications documentation

Concomitant medications will not be systematically collected on the case report form; however some relevant medications taken just before and during the treatment period will be captured in the study database. Additionally, medications that relate to glucose control will be captured from just prior to treatment through completion of the study period.

8.1.3 Vital signs

Routine vital signs per AHA guidelines for acute stroke patients will be followed.

8.1.4 NIHSS

The NIHSS will be obtained prior to randomization (baseline) by an investigator who is NIHSS certified. The NIHSS will be repeated at least once daily during the treatment period, and it is strongly recommended that this be completed by a certified investigator. It is required that the Day 90 NIHSS is scored by an NIHSS certified investigator.

8.1.5 Neuro Worsening Assessment

Any clinically relevant neurological worsening will trigger a clinical assessment including an NIHSS score. The SHINE study definition of neurological worsening will be considered any clinical change that is associated with a ≥4 point increase on the NIHSS score.

8.1.6 Protocol Deviation Documentation

Protocol deviations will be assessed throughout the study period and will be captured in WebDCU™. The details of identifying and reporting protocol deviations are described in the MOP.

8.2 Treatment Procedures

8.2.1 Decision Support Tool set up

Study laptops capable of internet connection will be supplied to all participating sites. All appropriate site personnel will be trained in the use of the tool. The decision support tool and control accounts are housed and run on a central dedicated secure server and are not resident on the laptop. The laptop serves as a convenient portal to access the central system. The FDA cleared computerized decision support tool will simplify and streamline study procedures both for bedside nurses and local study teams, and thereby substantially minimize possibilities for protocol deviations. Details of the decision support tool can be found in the MOP.

8.2.2 Blinding Set Up

- The SHINE trial is single blinded to the subject throughout the study (the treating team will be unblinded to treatment) and then is double blinded to include the examiner for the 90 day efficacy outcome assessment.
- Patients in both groups will receive an IV treatment (insulin or saline) and subcutaneous treatment (insulin or saline).

8.2.3 Drug Dosage/ Drug Administration

8.2.3.1 Control group protocol

IV Saline with Subcutaneous Insulin Injections Target Blood Glucose <180 mg/dL

8.2.3.1.1 Subcutaneous Insulin Sliding Scale and IV Saline (Placebo) for SHINE Control Group

Table 4. Subcutaneous Insulin Sliding Scale and IV Saline (Placebo) for SHINE Control Group				
Check finger stick glucose q 1 hr for the first 4 hours, then q 3 hrs (3:00, 6:00, 9:00, 12:00, 15:00, 18:00, 21:00, and 24:00), but give SQ insulin if indicated only 4/day (6:00, 12:00, 18:00, and 24:00)				
IV Saline	**SQ Human Regular Insulin (Humulin R or Novolin R) Sliding Scale**			
ml/hr	Glucose (mg/dL)	**Level 1** Insulin dose (units)	**Level 2** Insulin dose (units)	**Level 3 One time SQ basal insulin and continue Level 2** Insulin dose (units)
5	>450	8	16	16
	400-450	7	14	14
	351-399	6	12	12
	300-350	5	10	10
	251-299	4	8	8
	200-250	3	6	6
	180-199	2	4	4
4	80-179	0	0	0
0	<80	**See hypoglycemia protocol below (Section 8.2.3.3)**		

8.2.3.1.2 IV Saline Instructions (as per Table 4)

Patients randomized to the control group will receive continuous IV saline.

The IV saline will be adjusted using the glucose concentration from each finger stick check according to Table 4. This will include glucose checks at least every 1 hour for the first 4 hours and then every 3 hours for the remainder of the treatment protocol.

A hypoglycemia protocol will be initiated for any patient whose glucose concentration drops below 80 mg/dL (see Section 8.2.3.3).

The details of the IV saline protocol are provided in the MOP.

8.2.3.1.3 SQ Human Regular Insulin Sliding Scale Instructions (as per Table 4)

Patients randomized to the control group will receive subcutaneous insulin only up to four times a day as needed according the sliding scale in Table 4.

The glucose concentration will be checked using finger stick testing at least every 1 hour for the first 4 hours and then every 3 hours for the remaining treatment period as per Table 4. The subcutaneous insulin will only be given at 6:00, 12:00, 18:00 and 24:00 as needed.

All patients will start at Level 1 sliding scale dosing as shown in Table 4. If glucose concentrations on that dosing schedule are not adequately controlled (<180 mg/dL), patients will begin Level 2 on the second day. If they are still not adequately controlled at Level 2, they will advance to Level 3 on the third day. Level 3 treatment will include basal insulin (glargine, Lantus) at a dose of 40% of previous day's entire insulin dose and then continuation of the Level 2 sliding scale dosing as shown in Table 4.

The details of the subcutaneous protocol are provided in the MOP.

8.2.3.2 Intervention group

IV Insulin with Subcutaneous Meal Insulin or Saline Injections
Target Glucose Concentration 80-130 mg/dL

8.2.3.2.1 IV Insulin Instructions

Patients randomized to the intervention group will receive continuous IV insulin.

The decision support tool will require finger stick glucose checks at least every 1 hour for the first 4 hours and then approximately every 1 to 2 hours for the remainder of the treatment period.

The dosing will be recommended by the decision support tool. The required timing of the glucose check will also be indicated by the decision support tool. The decision support tool uses information about the specific patient to make the recommendation of best dosing to keep the glucose concentration in target.

A hypoglycemia protocol will be initiated for any patient whose glucose concentration drops below 80 mg/dL (see Section 8.2.3.3).

The details of the decision support tool and the insulin infusion dosing are provided in the MOP.

8.2.3.2.2 SQ Insulin and Saline Instructions

Subcutaneous injections will be given at meal time up to three times a day for patients who are eating.

Patients who are not eating or are receiving continuous tube feeds will receive placebo saline subcutaneous injections 2/day at 9:00 and 21:00 to simulate the 0-4 SQ insulin injections in the control group.

The details of the subcutaneous insulin/saline dosing are provided in the MOP.

8.2.3.3 Hypoglycemia Protocols for Control and Intervention Groups

Hypoglycemia protocol (Glucose concentration <80 mg/dL)

All insulin therapy will be stopped in the event that glucose falls below 80 mg/dL and the following protocol will be initiated:

- Stop all IV insulin and hold all subcutaneous insulin (stop all IV saline and saline subcutaneous injections as well).
- Stat draw of a serum sample is to be sent to the laboratory for confirmation if glucose <70 mg/dL.
- Glucose administration
  - Control Group – A dose of IV D50 25 ml (1/2 amp D50) will be given until blood glucose is ≥80 mg/dL.
  - Intervention Group – A dose of IV D50 will be given. The specific dose will be determined by the decision support tool based on the glucose concentration.
- Recheck blood glucose in 15 minutes, and repeat treatment every 15 minutes if needed until glucose is ≥80 mg/dL.
- Once glucose is ≥80 mg/dL:
  - Restart IV insulin or saline per protocol
  - Restart SQ insulin or saline per protocol

The details of the hypoglycemia protocol are provided in the MOP.

Severe Hypoglycemia (Glucose <40 mg/dL)

All glucose measurements <40 mg/dL will be captured as severe hypoglycemia, and measurements <70 mg/dL will be captured as hypoglycemia. Each event will be characterized as symptomatic or asymptomatic based on symptoms and signs determined by the hypoglycemia assessment tool described in the MOP. All spontaneous symptomatic complaints will trigger a stat glucose measure.

Nonverbal patients will be assessed for only the physiologic signs of hypoglycemia.

8.2.3.4 Suggested Care Post Study Treatment (based on ADA guidelines)[53,54]

At the completion of study treatment, the hospital treating team (not the study team) will determine the best long-term glucose control therapy for each patient. Investigators will be encouraged to follow the most recent ADA guidelines.

8.2.4 Concomitant or Ancillary Therapy

Throughout the study period, all AHA guidelines for acute stroke care[8] will be followed. This includes standard care for acute ischemic stroke patients such as swallowing evaluation prior to initiation of PO intake, DVT prophylaxis and early secondary stroke prevention therapy. Blood pressure and temperature recommendations also will be followed. Rehabilitation evaluation will be considered for every subject.

8.3 Clinical Guidelines

As above, the AHA acute ischemic stroke guidelines for standard clinical care will be followed for all subjects.

8.4 Follow-up Procedure

The goal of the study is to achieve complete, accurate follow-up for the three month study period. Subjects will be contacted at 6 weeks by phone for reports of SAEs, to capture early follow up information and to make arrangements for the 3 month study follow up visit during which time the primary clinical outcome (mRS) and secondary outcomes will be captured. Any subject unable to return in person will undergo a 3 month study follow up visit by phone.

8.5 Notification of Death

For each death, an AE form must be completed documenting the cause of death. An event that leads to death is always an SAE, although it may not be related or unexpected. The underlying cause of death will be required to be reported. Concurrent disease processes such as sepsis, pneumonia, etc. should be investigated. Within 24 hours of the discovery of a treatment related death, the

site should submit the information into the AE case report form. This entry will trigger an automatic e-mail notification to the independent safety monitor. A written report containing all relevant clinical information will be submitted to WebDCU™ within 5 calendar days of discovery of the death. This information will be reported to the sites for submission to their IRBs and submitted to the DSMB.

8.6 Procedure for Unblinding

Unblinding will not be necessary in the SHINE trial as the treating team will be unblinded to subject treatment. The 6 week follow-up phone call and the 90 day outcome assessment will be performed by a blinded study investigator and a blinding/unblinding survey will be completed by the subject and the investigator at the completion of the 3 month visit.

8.7 Schedule of Events

Table 5. Abbreviated Schedule of events in SHINE trial

Event	Screen*	Baseline*	Up to 3 days of Rx	Complete Rx/ D/C**	6 wks	90 days
Informed Consent	x					
Eligibility Assessment	x					
Randomization		x				
Study drug given		x ---	------			
Finger stick glucose check	x	x ---	------			
mRS[55], BI[13]					x	x
NIHSS[12]		x	x (daily)	x		x
SSQOL[14]						x
SAE/AEs*		x ---	------	-------	-----	x
Protocol Deviations		x ---	------	------	------	x
Neuro worsening		x ---	------	-------		
Lacunar subtype				x		

*****Screening is during eligibility assessment; baseline is at time of randomization. AEs will be followed through the treatment period only. SAEs will be reported throughout the study.

**Completion of 72 hr treatment period unless discharged prior to 72 hrs

9. DISCONTINUATION OF PARTICIPATION

9.1 Subject Removal from Therapy

As participation in the SHINE trial is voluntary, the subject or LAR may decline study therapy at any time. In addition, the treating physician may stop the study therapy if there is a safety concern. The subject will continue to be followed through the 90 Day visit unless informed consent is withdrawn.

9.2 Subject Withdrawal

The subject has the right to voluntarily withdraw from the study at any time for any reason without prejudice to his/her future medical care by the physician or at the institution.

For the occasional subject who withdraws consent, the date and reason for consent withdrawal should be documented. Subject data will be included in the analysis up to the date of the consent withdrawal.

A distinction will be made between subjects who fail to complete all forms on schedule or who miss some follow up assessments and the withdrawal of consent. Missed or rescheduled visits will be documented, but the subject will continue to be followed in the future according to protocol requirements, and all follow-up data will be included in the analysis.

9.3 Procedure for Discontinuation

The procedure to be followed at the time a subject/LAR withdraws consent from the trial:

(1) Check for the development of adverse events.

(2) Complete the End-of-Study form and include an explanation of why the subject is withdrawing.

(3) Subject or LAR will be required to document in writing his or her desire to withdraw.

9.4 Subject Lost to Follow-Up

In the event that all possible attempts to locate the subject have failed, a formal letter will be submitted to the Executive Committee (EC). The letter must document all efforts made by the investigator to contact the subject. When the EC is satisfied that all methods have been tried and have failed, the subject will be coded as lost to follow-up and the delinquency status will be removed.

9.5 Subject Transfers

Whenever a subject's medical care transfers to another clinical setting, every attempt must be made to obtain continued follow-up data and information on self- administered forms. The study coordinator should be notified immediately when this occurs, so that appropriate arrangements can be made, wherever possible for the subject to continue to participate in the study.

10. OUTCOMES DEFINTIONS

10.1 Primary

The primary outcome is the severity adjusted 90-day mRS. "Favorable" outcome is defined as mRS score of 0 in subjects with baseline NIHSS of 3-7, mRS of 0-1 in subjects with baseline NIHSS of 8-14, and mRS of 0-2 in subjects with baseline NIHSS of 15-22.[9, 10] All NETT and non-NETT investigators will be trained and certified in the same manner.

10.2 Secondary

- NIHSS
- Barthel Index (BI)
- Stroke Specific Quality Of Life (SSQOL) scale

Favorable outcomes for the NIHSS and BI are defined as: a score of 0-1 on the NIHSS and 95-100 on the BI. The SSQOL will be analyzed as a continuous outcome.

11. DATA MANAGEMENT

11.1 Data Processing

Data management will be handled by the NETT SDMC which is housed in the Data Coordination Unit (DCU) in the Division of Biostatistics and Epidemiology at the Medical University of South Carolina (MUSC). All study activities will be conducted in coordination with the study co-PIs, the hubs/spokes, and the NETT CCC and SDMC, and will use an electronic data acquisition method where all clinical data on enrolled subjects will be entered by the hub/spoke personnel in real time. The latest version of each CRF will be available as a PDF file on the study website for use as worksheets and source documents by study personnel.

The study data will be managed (including data queries) by the SDMC using the WebDCU™ system. This user-friendly web-based database system, developed by the SDMC, will be used for regulatory document management, subject randomization, data entry, data validation, project progress monitoring, subject tracking, user customizable report generation and secure data transfer.

11.2 Data Security and Confidentiality

During the course of the trial, user access to the files with subject identifiers, treatment assignment and files with study outcomes will be restricted to core staff with any exceptions to be approved by the Executive Committee.

In addition to use of passwords and other security measures, all documents containing identifying information on individuals or physicians are considered confidential materials and will be safeguarded to the greatest possible extent. No information, which identifies a specific person, hospital, or physician, will be released to, or discussed with anyone other than study staff members.

Because the SDMC uses a web-based system, source documents and CRFs will remain at the participating sites. The study database only identifies study subjects

by unique study identification codes. All data will be stored in a manner that is HIPAA compliant, without the ability to track the information back to a specific subject except through a password protected system. All collected information about a subject will be stored by a unique identification code. All SDMC personnel are certified by the NIH Office of Human Subjects Research in the Protection of Human Research Subjects course.

11.3 Data Quality Assurance

Upon entry of CRFs into the study database, quality control procedures will be applied at each stage of data handling in order to ensure compliance with GCP guidelines, integrity of the study data and document processing system reliability.

All sites will be monitored by the CCC and monitors will review source documents and case report form information. A quality assurance record audit will be implemented. Audit findings will be used to identify and correct problems in data collection.

12. STATISTICAL CONSIDERATIONS

12.1 Sample Size and Power Estimation

The primary outcome variable is the overall proportion of subjects experiencing a favorable outcome 90-days post randomization, where favorable is defined by the dichotomized mRS score as adjusted to the baseline NIHSS (stroke severity). Our preliminary data suggest a 25% rate of favorable outcome in the control group at 90 days is reasonable. A clinically relevant absolute difference in success rates between the two interventions is chosen as 7% (see preliminary data) (success $rate_{control}$=25%; success $rate_{infusion}$=32%). If the IV insulin group does not have at least a 7% or higher success rate than the control group, then IV insulin within 12 hours of symptom onset will not be considered a worthwhile therapy for hyperglycemic acute ischemic stroke subjects.

Based on the above information and taking into consideration the planned interim analyses, the study is powered to assure 80% likelihood of identifying a difference in success rates greater than or equal to 7%. Sample size estimation is based on the comparison of independent proportions. This estimation approach is conservative as the proposed regression analysis is generally more powerful than chi square test on individual outcomes. The maximum sample size required for randomization is 1314 subjects (657/treatment group). Although every attempt will be made to avoid drop outs and losses to follow up, the required sample size is inflated for a 3% non-adherence rate. The 3% rate is consistent with pilot data and the ALIAS study rates. The estimated total of subjects required is a maximum of 1400. The analysis plan does include a sample size re-estimation so this number could potentially change. Details of the re-estimation are in the Statistical Analysis Plan (SAP).

12.2 Statistical Analyses

12.2.1 Interim Analysis

Details of all planned statistical analyses including the interim analyses will be provided in the study SAP. In summary, four interim analyses for futility and overwhelming efficacy are planned. These analyses will use the error-spending function method with stopping guidelines that are similar to the O'Brien and Fleming (OBF) type stopping boundaries. The proposed timelines can be altered based on the input from the DSMB. The SDMC will conduct these analyses and compile the reports for the DSMB.

12.2.2 Interim Safety Analysis

The safety monitor and DSMB will receive periodic safety reports of all adverse events and serious adverse events. Statistical monitoring for safety will be limited to severe hypoglycemia (<40 mg/dL) during the treatment period and death rate within 90 days post randomization. Details on this monitoring plan are in the SAP.

12.2.3 Primary Efficacy Analysis

The primary outcome analysis of the 90-day mRS will use a stratified dichotomy methodology for assessing improvement as defined in the Primary Outcome section above. Outcome differences will be analyzed under the intention-to-treat principle, therefore all randomized subjects will be included in the primary analysis sample. To assess efficacy, the treatment groups will be compared with respect to the proportion with favorable outcome 90 days post randomization after controlling for the prognostic variables included in the randomization scheme. A Wald chi- square test will be performed to compare the treatment group proportions using a two-tailed significance level of 0.05. Adjusted relative risks will be reported with two-sided 95% confidence intervals.

Additional analyses will identify potential confounding (prognostic) variables to be used as covariates in subsequent secondary analyses of the primary outcome using generalized linear modeling techniques. Specific covariates include age, gender, race, ethnicity, admission blood glucose, previous stroke, time between stroke onset and treatment and lacunar subtype. If statistically significant differences are evident, post-hoc analyses will be conducted to determine if differences had an effect on the conclusions from the pre-specified primary analysis.

12.2.4 Secondary Analyses

This study is designed to test the primary hypothesis. However, it also offers the opportunity to conduct analyses to evaluate important additional neurological and functional outcomes using the NIHSS, BI and SSQOL. The secondary analyses will be conducted using the intention to treat population. In addition to these secondary clinical outcomes, the analysis

will include time to randomization from symptom onset and protocol conduct metrics between treatment arms: time to target, time in target, time to treatment and adherence to the decision support tool.

12.2.5 Safety Outcome Analyses

In addition to the continual monitoring of adverse events by the safety monitor and DSMB and the planned statistical monitoring for safety, final analyses of specified safety outcomes will be conducted as specified in the SAP.

13. ADVERSE EVENTS

13.1 Adverse Events

Adverse event reporting and procedures are described in detail in the MOP.

Adverse events will be defined and severity graded according to the Common Terminology Criteria for Adverse Events, (CTCAE), found in the MOP. AEs will be submitted online through WebDCU™ and coded centrally using MedDRA. Guidelines for report content and structure will be provided in the MOP. All adverse events occurring during treatment and all serious adverse events occurring during the study will be recorded. The site PI or Study Coordinator is responsible for entering any and all AEs and SAEs into the database and updating the information (e.g., date of resolution, action taken) as needed.

Study personnel will evaluate subjects while in the hospital and at each telephone communication and follow up for the presence of AEs (during treatment only) and SAEs.

13.2 Clinically Important Adverse Events

The following adverse events should be handled as serious.

- Neurological worsening lasting greater than 24 hours and associated with glucose concentration of <55 mg/dL.
- Severe Hypoglycemia (glucose <40 mg/dL).

13.3 Adverse Event Exceptions

Death due to the natural history of ischemic stroke will be recorded as a non- related serious adverse event. Additionally, all serious but known complications of stroke (i.e. malignant brain edema) will be recorded as non-related serious adverse events.

13.4 Obligation of Investigator

At the time of enrollment, an assessment of ongoing medical conditions and/or signs or symptoms will be recorded. Upon initiation of study protocol, all adverse events, whether or not attributed to the study intervention, observed by the Investigator or reported by the subject, will be recorded on the appropriate Case

Report Forms. Attributes will include a description, date of onset and resolution, duration, severity, assessment of relatedness to the intervention, action taken, and outcome. All adverse events must be followed to resolution or until the end of the study treatment period, whichever comes first.

If the adverse event is sufficiently severe, the investigator is obligated to conduct a termination assessment, and if appropriate, should halt study treatment. The subject should be provided with medical supervision and appropriate treatment until symptoms cease.

13.5 Reporting Procedures

In order to assure prompt and complete reporting of adverse events or complications of intervention, the following general guidelines are to be observed.

All adverse events, whether serious or not, will be collected through completion of study therapy. Adverse events will be captured by the treating team and the study team based on history, physical exam, medical records and laboratory findings. The independent safety monitor will review non serious AEs regularly.

Serious adverse events (SAE) will be captured throughout the 3 month study period. All SAEs, including those that are judged to be related to study therapy, and especially severe hypoglycemia, will be data entered by the site personnel into the study database within timelines defined in the MOP. Upon data entry, the system will trigger an automatic e-mail notification to the independent Medical Safety Monitor (MSM) stating that an SAE has occurred. The MSM will access the information via the password protected web based system for review and report safety concerns to the DSMB.

14. INVESTIGATIONAL DRUG DESCRIPTION

The investigational drug is insulin which is FDA approved for the treatment of hyperglycemia. Therefore, it will be labeled, stored, prepared and distributed through the hospital pharmacy as it otherwise would. Blinding procedures (to maintain the subject blind) will take place and are described in detail in the MOP.

15. REGULATORY AND ETHICAL OBLIGATIONS

15.1 Informed Consent

In accordance with US FDA regulations (21 CFR 50) and guidelines (Federal Register, May 9, 1997, Vol. 62, Number 90 - ICH Good Clinical Practice Consolidated Guideline) it is the investigator's responsibility to ensure that informed consent is obtained from the subject or subject's LAR before participating in an investigational study, after an adequate explanation of the purpose, methods, risks, potential benefits and subject responsibilities of the study. Procedures that are to be performed as part of the practice of medicine and which would be done whether or not study entry was contemplated, such as for

diagnosis or treatment of a disease or medical condition, may be performed and the results subsequently used for determining study eligibility without first obtaining consent. On the other hand, informed consent must be obtained prior to initiation of any screening procedures that are performed solely for the purpose of determining eligibility for research.

Each subject/LAR must be given a copy of the informed consent. The original signed consent must be retained in the institution's records and is subject to review by the sponsor, DCU, the FDA or representative from another agency that performs the same function, and the IRB responsible for the conduct of the institution. All elements listed in the International Conference on Harmonisation Good Clinical Practice guidelines must be included in the informed consent.

Informed consent will be obtained by either the site Principal Investigator or by individuals approved by the site Principal Investigator and whose names have been submitted to the NETT Regulatory Database. Informed consent will be obtained from the subject or subject's LAR after the details of the protocol have been reviewed. The individual responsible for obtaining consent will assure, prior to signing of the informed consent, that the subject has had all questions regarding therapy and the protocol answered.

15.2 Institutional Review Board (IRB)

In accordance with US FDA regulations (21 CFR 56) and guidelines (Federal Register, May 9, 1997 Vol. 62 Number 90 - International Conference on Harmonisation Good Clinical Practice Consolidated Guideline) all research involving human subjects and changes to the research plan must be reviewed and approved by an IRB.

15.2.1 Initial Review and Approval

A copy of the protocol, proposed informed consent form, other written subject information, and any proposed advertising material must be submitted to the enrolling site's IRB for written approval. A copy of the IRB approval of the protocol and informed consent form must be submitted to the NETT Regulatory database and approved before recruitment of subjects into the study.

15.2.2 Amendments

Protocol amendments may only be made with the prior approval of Executive Committee. The site Principal Investigator must agree to, and obtain approval from the IRB, for all protocol amendments and revisions to the informed consent document. The site Principal Investigator should notify the IRB of serious adverse events occurring at the site and other adverse event reports recorded in the study database, in accordance with local procedures and Section 13.5 of this protocol. Copies of all approvals and approved versions of the informed consent must be submitted to the central regulatory document database housed within WebDCU™.

15.2.3 Annual Renewal

The site Principal Investigator will be responsible for obtaining annual IRB approval renewal throughout the duration of the study. Copies of the site Principal Investigator's reports and the IRB's continuance of approval must be submitted electronically to the central regulatory document database.

16. STUDY ORGANIZATION

16.1 Executive Committee

In addition to the SHINE leadership, the Executive Committee (EC) has overall responsibility for assuring the scientific, clinical and ethical integrity of the study. The executive committee will include the NINDS Project Officer, administrative core leadership, the NETT CCC leadership and SDMC leadership.

The details of the executive committee functions and membership are detailed in the MOP.

16.2 Data and Safety Monitoring Board

The NINDS-appointed DSMB will plan to meet semi-annually with interim conference calls as needed, to review interim data relating to the trial. The functions of the DSMB will include:

(1) Review of all adverse effects or complications related to the trial interventions;

(2) Review of interim data on the primary and secondary outcomes according to prespecified monitoring guidelines;

(3) Monitor accrual;

(4) Review summary reports relating to performance of centers relative to compliance with protocol requirements;

(5) Recommend to the Executive Committee that:

a) Trial continue as planned;

b) Trial be modified with justification of modification as DSMB deems appropriate;

c) Trial should be stopped with disclosure of basis for recommendation.

In general, clinical investigators will not be present at the Closed Sessions of the DSMB, but may on occasion be requested to present or provide information to assist the DSMB in carrying out its functions appropriately.

16.3 Ancillary Studies

General information on proposals for SHINE ancillary studies and detailed procedures for approved and funded ancillary studies can be found in the MOP. Sites will not be required to participate in any ancillary study that requires additional data collection, but they will be encouraged to participate in accepted studies.

16.3.1 Optional Insights on Selected Procoagulation Markers and Outcomes in Stroke Trial (I-SPOT) Ancillary Study

Participants who consent to the SHINE trial **may** be asked to consent to an optional ancillary study. Subjects who consent to I-SPOT will provide blood samples at 2 time-points (baseline and 48 hours). Those patients who decline the ancillary study may still participate in the SHINE trial.

The I-SPOT trial will recruit 315 SHINE patients. Blood coagulation marker levels will be measured before and at 48 hours after the start of treatment. Baseline and temporal changes in biomarkers levels will be compared between SHINE treatment groups. Details of I-SPOT can be found in the ancillary trial protocol and laboratory manual.

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