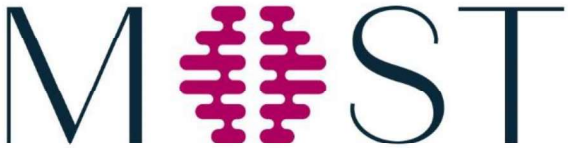

Protocol Title

Multi-arm Optimization of Stroke Thrombolysis (MOST): a single blinded, randomized controlled adaptive, multi-arm, adjunctive-thrombolysis efficacy trial in ischemic stroke.

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MOST

AGREEMENT ON THE PROTOCOL

By signing below I confirm that:

1) I have read this protocol and it contains all necessary details for conducting this study

AND

2) I agree to conduct the trial in compliance with this protocol and to adhere to all regulations that govern the conduct of the study.

_____ _____

Principal Investigator's Signature **Date**

_____

Principal Investigator's Printed Name

_____

Site Name

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STUDY SYNOPSIS

Study Title

Multi-arm Optimization of Stroke Thrombolysis (MOST) Stroke Trial

Objectives

The primary efficacy objective of the MOST trial is to determine if argatroban (100μg/kg bolus followed by 3μg/kg per minute for 12 hours) or eptifibatide (135μg/kg bolus followed by 0.75μg/kg/min infusion for two hours) results in improved 90-day modified Rankin scores (mRS) as compared with placebo in acute ischemic stroke (AIS) patients treated with standard of care thrombolysis (0.9mg/kg IV rt-PA or 0.25mg/kg IV tenecteplase or TNK) within three hours of symptom onset. Patients may also receive endovascular thrombectomy (ET) per usual care. Time of onset is defined as the last time the patient was last known to be well.

The primary safety objective of the MOST trial is to determine the safety of argatroban and eptifibatide in combination with IV rt-PA or TNK with or without ET per usual care, where safety is measured by symptomatic intracranial hemorrhage (sICH) within 36 hours from randomization.

Design and Outcomes

This is a three-arm, adaptive, Phase-3, single blinded, randomized controlled clinical trial at up to 110 sites in the United States and Canada. The first 150 subjects will be randomized 1:1:1. From 150-500 patients, response adaptive randomization (RAR) will favor the treatment arm showing the greatest benefit based on accrued data. After 500 patients, one or both intervention arms may be carried forward for fixed randomization versus placebo control. A maximum of 1200 patients will be enrolled.

The primary efficacy outcome for MOST will be the 90-day mRS translated into utilities, which measure cost-effectiveness and benefits of a given intervention from the patient's perspective. The primary safety outcome is sICH. Secondary outcomes include a comparison between treatment groups of: the proportion of participants with NIHSS ≤2 at 24 hours; change from baseline to 24-hour NIHSS; proportion with 90-day mRS 0 or 1 and 0- 2; 90-day ordinal analysis of the mRS; 90-day EQ-5D; the proportion of participants who have thrombectomy; the proportion of participants with parenchymal hemorrhage types 1 (PH-1) and 2 (PH-2); any ICH on brain imaging within 36 hours of randomization; major hemorrhage (defined as requiring >2 units packed red blood cells) other than intracranial hemorrhage within 7 days; 90-day all-cause mortality; evaluation of treatment effect in rt-PA and TNK subjects; evaluation of treatment effect in ET and non-ET subjects; and, evaluation of race/ethnicity and gender differences in treatment effect.

Interventions and Duration

After thrombolysis is started in eligible ischemic stroke patients, the patient or legally authorized representative (LAR) will be approached for participation in the study and consent obtained in eligible patients. Placebo, argatroban or eptifibatide will be started as soon as possible after consent is obtained. Participants should receive assigned study drug within 60 minutes of initiation of IV thrombolysis, but administration is allowed up to 75 minutes. A repeat NIH stroke scale will be performed at 24 (+/- 12) hours after initiation of thrombolysis. The primary safety outcome will be measured by any evidence of sICH within 36 hours from randomization as determined by a medical safety monitor and an independent neuroradiologist. Functional outcome at 90 days, the primary efficacy outcome, will be assessed by centralized video adjudication of interviews of the patient and/or LAR.

Sample size and Power

With a maximum N=1200, the design provides at least 80% power to detect the specified treatment effect (0.4 utilities) when only one active arm is effective. If both treatment arms are equally effective, power is 89%.

1 STUDY OBJECTIVES

1.1 Primary Objectives

The primary efficacy objective of the MOST trial is to determine if argatroban (100μg/kg bolus followed by 3μg/kg per minute for 12 hours) or eptifibatide (135μg/kg bolus followed by 0.75μg/kg/min infusion for two hours) results in improved 90-day modified Rankin scores (mRS) as compared with placebo in acute ischemic stroke (AIS) patients treated with standard of care 0.9mg/kg IV rt-PA or 0.25mg/kg IV TNK within three hours of symptom onset. Patients may also receive endovascular thrombectomy (ET) per usual care.

The primary safety objective of the MOST trial is to determine the safety of argatroban and eptifibatide in combination with IV thrombolysis with or without ET per usual care, where safety is measured by symptomatic intracranial hemorrhage (sICH) within 36 hours from randomization.

1.2 Secondary Objective

Secondary objectives include a comparison between treatment groups of: the proportion of participants with NIHSS ≤2 at 24 hours; change from baseline to 24-hour NIHSS; proportion with 90-day mRS 0 or 1 and 0-2; 90-day ordinal analysis of the mRS; 90-day EuroQol (EQ-5D), a self-administered questionnaire that is widely used to obtain utility values to derive quality-adjusted life years (QALY) for a given intervention; the proportion of participants who have thrombectomy; the proportion of participants with parenchymal hemorrhage types 1 (PH-1) and 2 (PH-2); any ICH on brain imaging within 36 hours of randomization; major hemorrhage (defined as requiring >2 units packed red blood cells) other than intracranial hemorrhage within 7 days; 90-day all-cause mortality; evaluation of treatment effect in rt-PA and TNK subjects; evaluation of treatment effect in ET and non-ET subjects; and, evaluation of race/ethnicity and gender differences in treatment effect.

2 BACKGROUND

2.1 Rationale

Intravenous (IV) thrombolysis is the only proven effective medication for the treatment of acute ischemic stroke (AIS). The aim of thrombolysis is recanalization and this occurs in ~50% of occluded arteries one hour after treatment. Arterial reocclusion occurs in 14-34% of thrombolysis treated patients within two hours and is associated with worse outcome. Overall, 50% of thrombolysis treated AIS patients are disabled at three months, likely owing to poor recanalization, reocclusion, or permanent neurological injury occurring before recanalization.[1-7]

Addition of endovascular thrombectomy (ET) to thrombolysis is a recent major advance for AIS patients with large vessel occlusions (LVO), further supporting the impact of recanalization on outcome.[8-12] However, only 46% of ET treated patients in published trials achieved functional independence (mRS 0-2) at 90 days[13,14]; 31% did not achieve good recanalization[13,14]; 25% had persistent occlusion at 24 hours[8]; and, only 7% of United States (US) hospitals perform ET.[15] Thus, there is an unmet clinical need for adjunctive IV medications that *could be administered at all hospitals (small and large) that treat patients with IV thrombolysis* to augment thrombolysis and reperfusion.

Thrombus formation and propagation are complex processes initiated by exposed collagen and/or tissue factor at the site of vascular injury. Recruitment and activation of platelets, platelet aggregation and cross- linking with fibrin(ogen) mediated by the glycoprotein (GP) 2b/3a receptor ensue, generating thrombin and fibrin. Thrombin further propagates platelet activation and aggregation in a positive feedback loop.[16] While the fibrin component of a thrombus may be sensitive to thrombolysis, the aggregated platelets resist dissociation and enhance thrombin generation.[17] Thrombolysis also stimulates platelet aggregation and thrombin generation, which may potentiate thrombosis and contribute to arterial reocclusion and neurological deterioration.[1-3,18] Two approaches that may augment thrombolysis and prevent arterial reocclusion are direct thrombin inhibition with argatroban and inhibition of the GP2b/3a receptor with eptifibatide.

Direct Thrombin Inhibition with Argatroban Augments Thrombolysis

Argatroban is a derivative of arginine that competitively binds to the active site of thrombin thereby preventing

fibrin deposition.[19] With a half-life of 30 minutes, argatroban has an immediate anticoagulant effect after IV administration which is rapidly reversed with discontinuation of the drug.[19] In murine models of middle cerebral artery (MCA) occlusion, argatroban reduced the formation of microthrombi, reduced lesion volume, and, when combined with recombinant tissue plasminogen activator (rt-PA), reduced fibrin deposition and extended the time-window for rt-PA.[20,21] A randomized controlled trial of single agent IV argatroban within 48 hours of stroke symptom onset at 60mg/day for two days and 10mg twice a day for five days resulted in improved symptoms and daily activities in the argatroban group.[22] A randomized placebo controlled safety study in 171 AIS patients found that high dose (100μg/kg bolus followed by 3μg/kg/minute, n=59) and low dose (100μg/kg bolus followed by 1μg/kg/minute, n=58) argatroban were safe when started within 12 hours of symptom onset and did not increase sICH rates (high- dose argatroban 5.1%; low-dose argatroban 3.4%; placebo 0%).[23] Based on these data, we conducted the Argatroban t-PA Stroke Study (ARTSS), ARTSS-2 and ARTSS-IA trials which found a favorable direction of effect with no safety concerns of argatroban plus rt-PA for AIS.

Inhibition of the GP 2b/3a Receptor with Eptifibatide Augments Thrombolysis

The final step of platelet aggregation is mediated via the GP2b/3a receptor.[17] Murine MCA occlusion model studies have investigated several GP2b/3a inhibitors combined with rt-PA and found that addition of GP2b/3a inhibitors to rt-PA prevented microvascular platelet aggregation, increased recanalization rates compared to rt-PA alone (50% vs. 20%) and reduced infarct volume by 25%.[24-26] Eptifibatide was specifically developed to ensure rapid inhibition of platelet aggregation (within 15 minutes), a short half-life (~2 hours) and rapid dissociation from platelets with 50% restoration of platelet function within 2-4 hours of discontinuation.[17] In myocardial infarction, addition of eptifibatide to rt-PA increased TIMI grade 3 flow (66% vs. 39%, P=.006)[27] and reduced coronary events without increasing the rates of bleeding complications compared to rt-PA alone.[28,29] Based on these data, we conducted the Combined Approach to Lysis Utilizing Eptifibatide and rt-PA in Acute Ischemic Stroke (CLEAR), CLEAR-Enhanced Regimen (CLEAR-ER) and CLEAR-Full Dose Regimen (CLEAR-FDR) trials which found a favorable direction of effect with no safety concerns.

2.2 Supporting Information and Prior Clinical Experience

Overall, six completed Phase 2 trials suggest the combination therapies are safe at the doses proposed. The studies were underpowered to demonstrate efficacy, but our analyses suggest a direction of effect in favor of the combination therapies.[30-35] We propose to study this definitively in the MOST trial.

Table 1 – Design and Sample Size of Six Completed Phase 2 Trials

	ARTSS	ARTSS-2	ARTSS-IA	CLEAR	CLEAR-ER	CLEAR-FDR
Intervention	0.9mg/kg rt-PA + low dose argatroban	0.9mg/kg rt-PA + low or high dose argatroban	0.9mg/kg rt-PA + high dose argatroban	0.3mg/kg and 0.45mg/kg rt-PA + eptifibatide	0.6mg/kg rt-PA + eptifibatide	0.9mg/kg rt-PA + eptifibatide
Study Size	n=65, single arm	n=90, 3-arms	n=10, single arm	n=94, 69 combination, 25 rt-PA	n=126, 101 combination, 25 rt-PA	n=27, single arm
Randomized	No	Yes	No	Yes	Yes	No

The ARTSS Trial – Low Dose Argatroban + Standard rt-PA is Safe and Promising

This single arm study of 65 patients combined standard dose rt-PA with 100μg/kg bolus of argatroban followed by a 48-hour infusion targeting a partial thromboplastin time (PTT) of 1.75 x baseline. The mean ± SD age for ARTSS patients was 63 ± 14 years and the median (range) NIH stroke scale score (NIHSS) was 13 (3-25). The endpoints were sICH and recanalization rates. sICH rate was 4.6% and 78% achieved partial or complete recanalization at 24 hours.[30] Ultra-early recanalization at 2-hours post rt-PA was measured using validated and centrally adjudicated transcranial Doppler (TCD) ultrasound waveforms.[36] Although this was a single-arm study, eligibility criteria and outcome variables were *a priori* chosen to closely match the CLOTBUST study[18] that included a control arm (rt-PA alone) to allow comparison of 2-hour recanalization. Complete recanalization at 2 hours occurred in 30% of argatroban + rt-PA patients compared to 13% rt-PA alone.

The ARTSS-2 Trial – Higher Dose Argatroban + Standard IV rt-PA is Safe and Promising

This Phase 2 randomized controlled clinical trial was designed to estimate overall treatment benefit among stroke patients treated with rt-PA who are randomized to either low dose argatroban (100μg/kg bolus, followed

by 1µg/kg/minute IV infusion for 48 hours), high dose argatroban (100µg/kg bolus, followed by 3µg/kg/minute IV infusion for 48 hours) or rt-PA alone. ARTSS-2 enrolled 90 patients at seven US and seven United Kingdom (UK) centers. Characteristics of enrolled patients are shown in Table 2. More argatroban patients reached mRS of 0-1 at 90 days as shown in Figure 1a and a trend in favor of improved outcomes in the high dose argatroban arm is shown in Figure 1b along with similar sICH.[31] Relative risks were adjusted for clinical site, terminal ICA occlusion, and Hemorrhage After Thrombolysis (HAT) score[37] which includes NIHSS, glucose and CT hypodensity. The equivalent difference in the utility score in favor of the high dose argatroban combination arm would be 1.0 (-0.9, 2.8).

Table 2 - ARTSS-2 Patient Characteristics

	IV rt-PA Alone *N=29*	Low Dose Argatroban *N=30*	High Dose Argatroban *N=31*
Age in years, mean ± SD	68.9 ± 15.4	70.9 ± 15.1	67.1 ± 13.4
Stroke Onset to IV rt-PA bolus, minutes, mean±SD	110.8 ± 44.7	132.3 ± 51.8	115.6 ± 46.2
Baseline NIHSS, (median, range)	15 (4-26)	16 (2-29)	13 (3-33)
ASPECTS score, (median, IQR)	10 (8, 10)	8 (6, 10)	9 (8, 10)

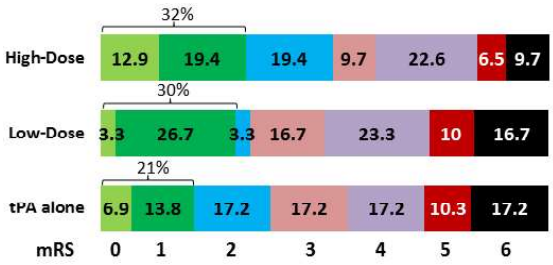


Figure 1a – ARTSS-2 distribution of 90-day mRS

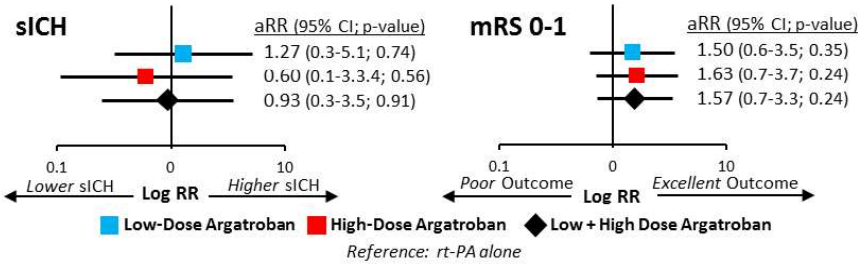


Figure 1b – ARTSS-2 adjusted RR for sICH

The ARTSS-IA Trial – rt-PA + Argatroban + ET is Safe

This proof-of-concept, single-arm, feasibility and safety study (ARTSS-IA; NCT02448069) assessed the MOST high dose argatroban regimen in AIS patients who receive IV rt-PA and ET. Ten patients were enrolled. Median age was 68 years (range 52-92) and median NIHSS was 19.5. All 10 patients received 12 hours of argatroban infusion and none suffered sICH or procedural complications. Study procedures did not prolong ET time metrics (i.e., imaging to groin-puncture) compared with non-ARTSS-IA ET cases.[32]

The CLEAR Trial – Half Dose rt-PA + Eptifibatide is Safe

This randomized dose escalation study showed that eptifibatide (75µg/kg bolus followed by 0.75µg/kg/min infusion for 2 hours) could be safely combined with 0.3mg/kg or 0.45mg/kg of rt-PA administered within three hours of symptom onset in AIS. sICH rate was 1% with no signal of efficacy as compared to IV rt-PA alone.[33]

The CLEAR-ER Trial – 0.6mg/kg rt-PA + Eptifibatide is Safe and Promising

CLEAR-ER randomized 126 AIS patients treated with rt-PA within three hours to 0.6mg/kg rt-PA plus eptifibatide (135µg/kg bolus and a 2-hour infusion at 0.75µg/kg/min) (n=101) vs standard rt-PA (0.9mg/kg) (n=25). Combination arm patients were non-significantly younger (72 vs 76 year, P=0.63), had lower NIHSS (12 vs 17, P=0.11) and had shorter times to IV rt-PA (113 vs 129 minutes, P=0.69). The age, NIHSS and time- to-IV rt-PA adjusted odds ratio was 1.38 (95% confidence interval (CI), 0.51-3.76; P=0.52) in favor of rt-PA +eptifibatide.[34] The sICH rate was 2%.[34]

Due to imbalances in the treatment and control arms of CLEAR-ER, we also matched 85 CLEAR-ER to 169 contemporaneous IMS III/ALIAS rt-PA only subjects using a propensity score matching approach.[38] The primary outcome was 90-day severity-adjusted mRS dichotomization based on baseline NIHSS.[39] Median age in CLEAR-ER and control subjects was 68 years; median NIHSS in the CLEAR-ER subjects was 11 and in control subjects 12. At 90 days, 45% of CLEAR-ER

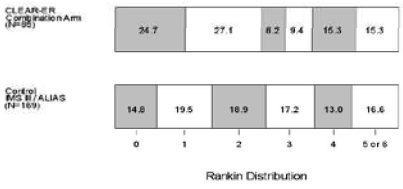


Figure 2 – mRS

subjects had favorable outcomes vs 36% in controls (unadjusted RR 1.24, 95% CI 0.91-1.69, p=0.18) (Figure 2). Secondary outcomes were 52% vs 34% excellent outcomes (mRS 0-1) (RR 1.51, 1.13-2.02, p=0.007); 60% vs 53% favorable outcome (mRS 0-2) (RR 1.13, 0.90-1.41, p=0.31); and ordinal Cochran-Mantel-Haenszel p=0.10.[38] The equivalent difference in the utility score in favor of the eptifibatide combination would be 0.43 (-0.37, 1.24).

The CLEAR-FDR Trial – Standard rt-PA + Eptifibatide is Safe and Promising

This was a single arm safety study of standard dose (0.9mg/kg) rt-PA plus eptifibatide. The primary goal was to ensure with high probability (defined as 80%) that the rate of sICH does not exceed 8%, the expected sICH rate based on patients from the NINDS trial who meet inclusion/exclusion criteria for the CLEAR trials. The stopping rule for the CLEAR-FDR trial was 3 sICH cases within the first 19 patients enrolled or 4 out of 29 patients enrolled. With a sample size of 30, a two-sided 95% confidence interval for a rate of 8% would be 0% to 18%. Twenty seven AIS patients were enrolled. Median age was 73 years (range 34-85) and median NIHSS was 12 (range 6-26). One sICH (3.7%, 95%CI 0.7%-18%) occurred.[35] We found comparable safety of full dose rt-PA plus eptifibatide with historical rates of sICH with rt-PA alone.

We also performed propensity score matching of 18 CLEAR-FDR subjects and 52 rt-PA only subjects from both IMS III and ALIAS. All subjects had a baseline mRS of 0 or 1. At 90 days, CLEAR-FDR subjects had a nonsignificant greater proportion of patients with a favorable primary outcome (61% versus 38%; RR 1.59; 95% CI 0.96-2.63; P=0.10). Secondary outcomes also favored CLEAR FDR subjects: mRS 0-1 67% versus 38% (RR 1.73, 95% CI 1.08-2.79; P=0.04); mRS 0-2 67% versus 58% (RR 1.16, 95% CI 0.77-1.73; P=0.50); and ordinal Cochran-Mantel-Haenszel, P=0.13. The equivalent difference in the utility score in favor of the eptifibatide combination would be 1.14 (-0.55, 2.83).

2.3 Standard of Care Intravenous Thrombolysis – rt-PA or TNK

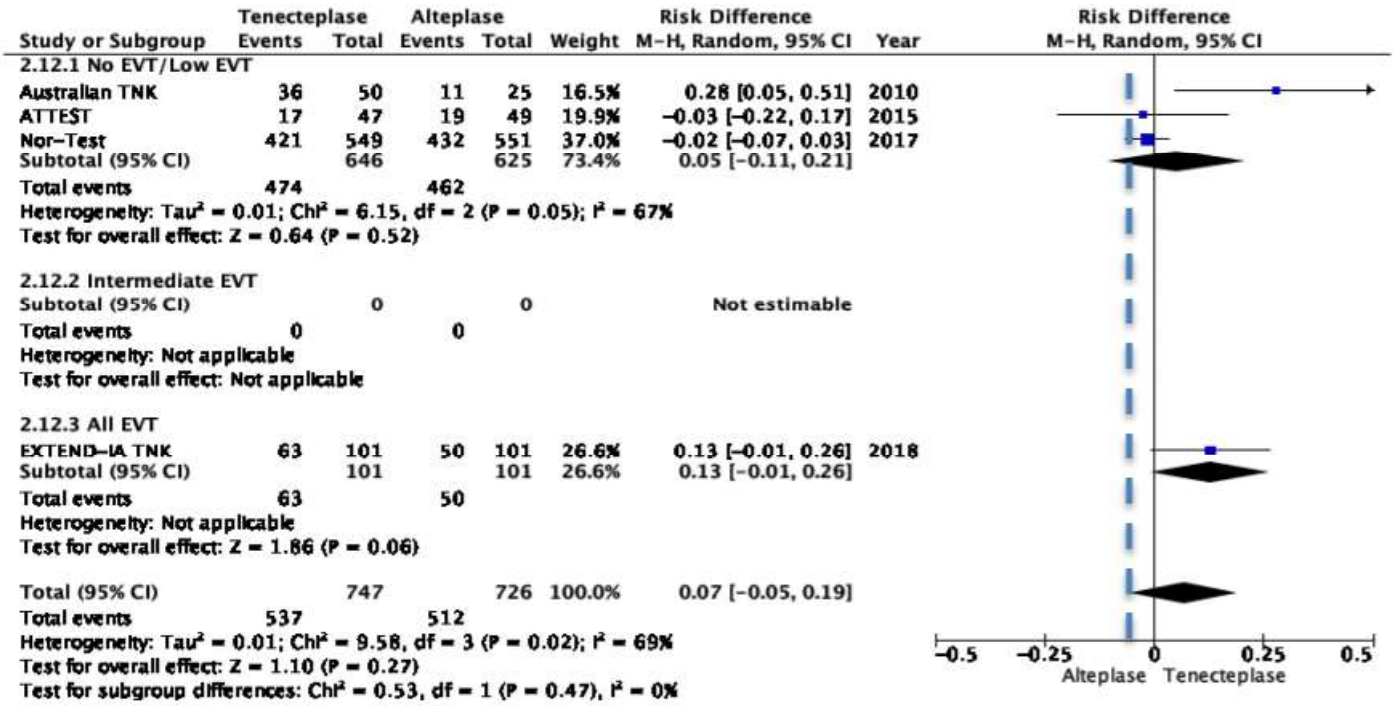
Treatment with IV rt-PA is an FDA approved medication for AIS. Emerging data on tenecteplase (TNK) has led to its adoption as standard practice globally and in the United States. rt-PA is administered as a bolus followed by an hour infusion while TNK, a variant of rt-PA, is a single bolus. rt-PA is cleared from the plasma with an initial half-life of 5 minutes, while the terminal half-life is 72 minutes. TNK is cleared from plasma with an initial half-life of 20 to 24 minutes, while the terminal half-life is 90 to 130 minutes.[40]

A meta-analysis of five randomized trials involving 1,585 patients demonstrated non-inferiority of TNK to rt-PA.[41] The American Stroke Association treatment guidelines indicate it may be reasonable to use TNK 0.25mg/kg as an alternative to alteplase in cases of patients with large vessel occlusion and more severe strokes undergoing thrombectomy (AHA Class 2b, Level of Evidence B-R).[42] Several large academic centers in the United States have switched to TNK as standard of care instead of rt-PA in all AIS patients.[43] Additionally, the Canadian Alteplase compared to Tenecteplase (AcT) trial involving 1600 patients demonstrated non-inferiority of TNK to rt-PA establishing it as a reasonable alternative and providing further rationale for global thrombolytic standards.[44] Thus, for the MOST trial, 0.25mg/kg TNK or 0.9mg/kg rt-PA within three hours of AIS symptom onset will be allowed as standard of care thrombolysis per local practice at performance sites.

TNK is Non-inferior with Similar Safety as rt-PA in AIS

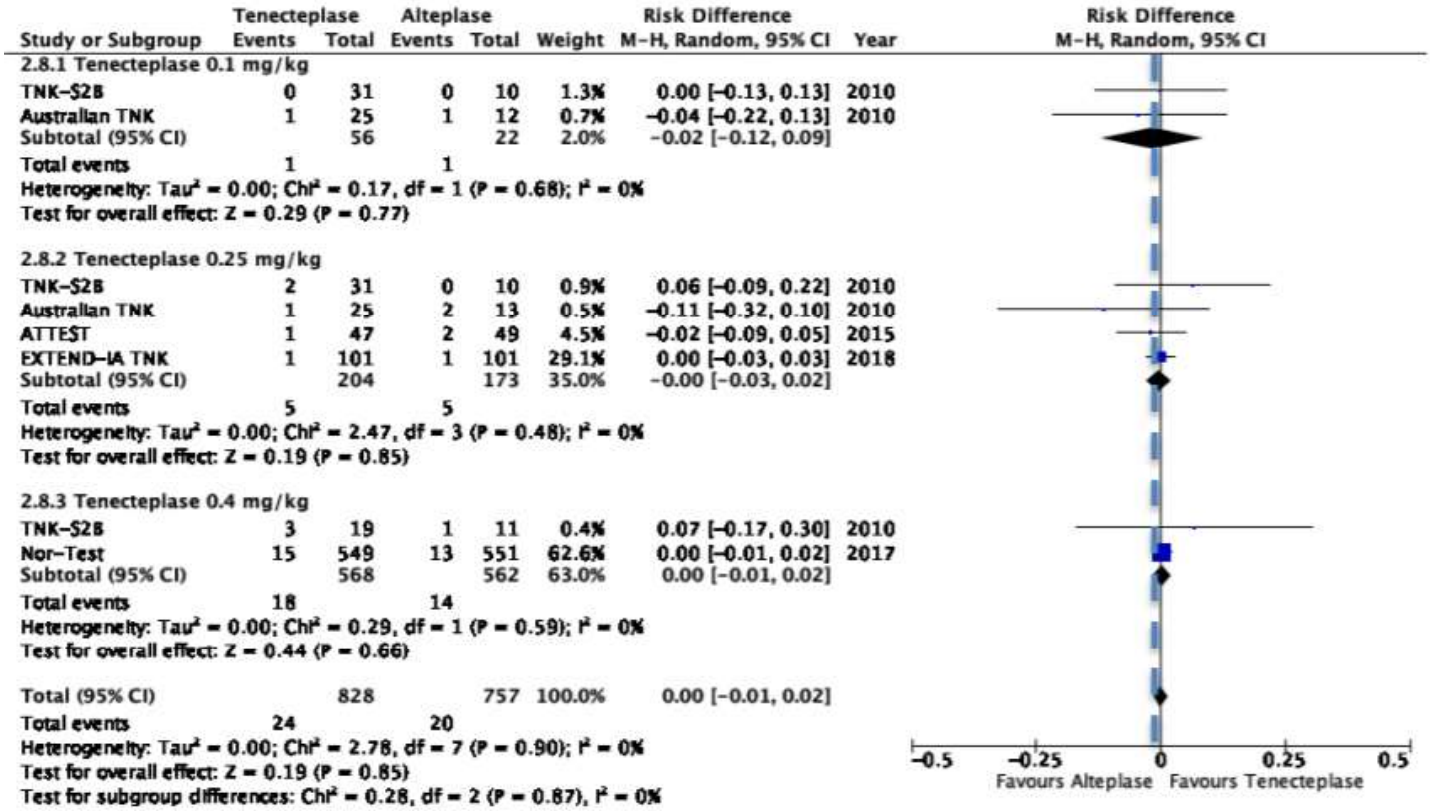
Forest plots from the meta-analysis by Burgos and Saver[41] below indicate non-inferiority/no difference in functional outcomes, symptomatic intracranial hemorrhage or mortality between rt-PA and TNK.

Figure 3 – Comparison of Functional Independence



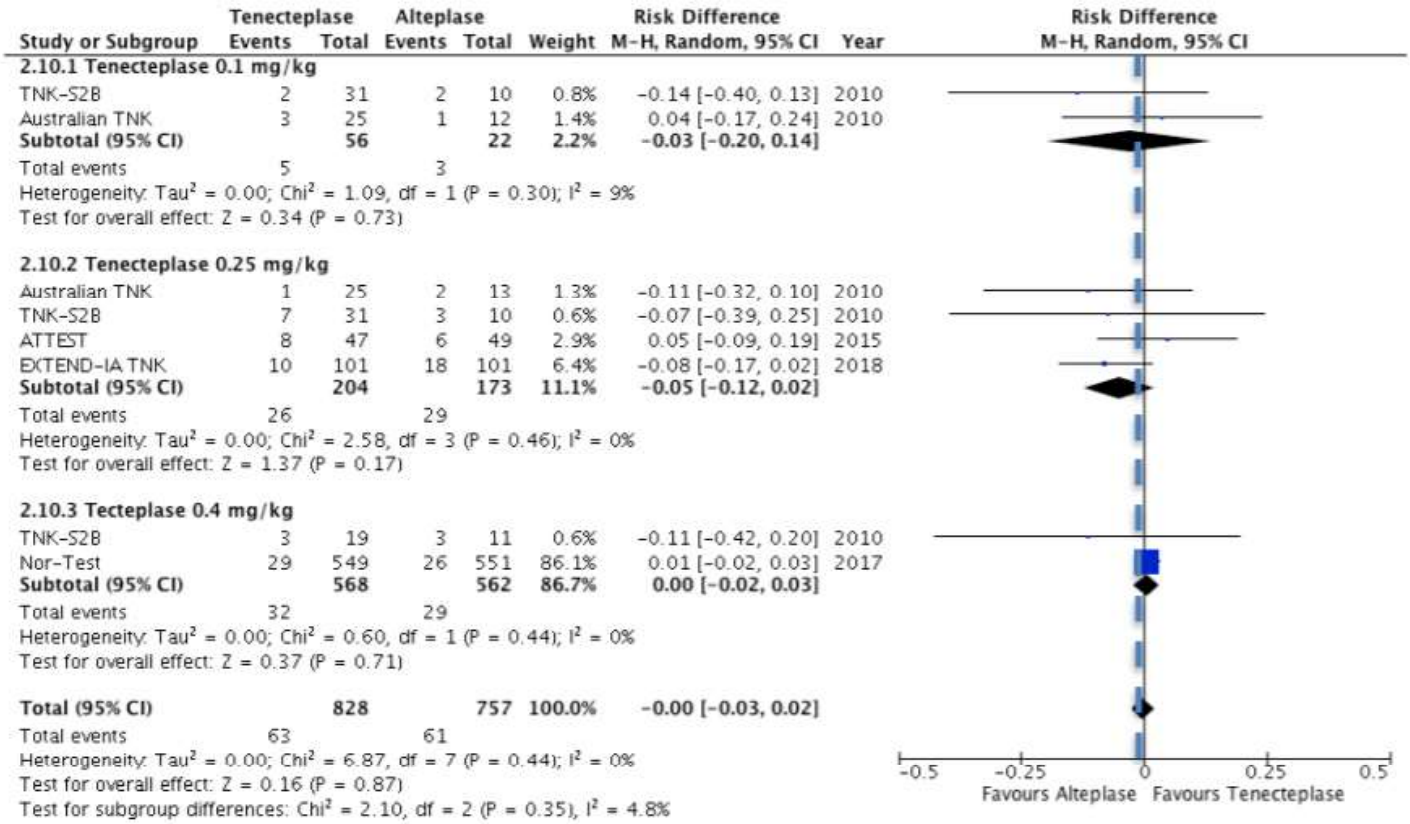
Forest Plot comparing Tenecteplase vs Alteplase, for functional independence (mRS 0-2), in trials with no or uncommon (0-10%) use of concomitant endovascular thrombectomy (EVT), intermediate levels (11-99%) of concomitant EVT, or planned EVT for all patients. There was no evidence of heterogeneity across subgroups, p(interaction) = 0.47, I2=0%.

Figure 4 – Comparison of Symptomatic Intracranial Hemorrhage



Forest plot comparing Tenecteplase by dose subgroups vs Alteplase, for the safety endpoint Symptomatic ICH. Overall, the risk difference point estimate favored TNK over ALT: 0% (95% CI -1% to 2%). The lower 95%CI bound of -1% fell on the margin. The dashed blue line indicates the -1% non-inferiority margin.

Figure 5 – Comparison of Mortality



Forest plot comparing Tenecteplase by dose subgroups vs Alteplase, for safety endpoint death. The risk difference point estimates did not favor TNK: 0% (95% CI -3% to 2%). The lower 95%CI bound of -3% did not fall within the stringent margin of -1%. Dashed blue line indicates the -1% non-inferiority margin.

Safety of Combining TNK with Argatroban or Eptifibatide in AIS

The existing preliminary data and Phase II trials support safety of the combination of rt-PA with the study interventions. Given the similarity in the safety profile of TNK and rt-PA in AIS, we do not expect safety concerns by combining TNK with argatroban or eptifibatide. However, there is limited data available. The safety plan for MOST allows controlled monitoring of subjects after every 30 enrollments. Specifically, within the TNK and rt-TPA subgroups, sICH rates by treatment group will be reported to the DSMB. The probability that observed sICH rates are higher than the expected 3% in any given combination arm will be estimated and DSMB recommendations will be followed. A 95% probability that the rate in an intervention arm or subgroup exceeds an expected rate triggers DSMB review. After review of data from every 30 enrollments, the DSMB may request a detailed review at lower probability at its discretion. Details of safety monitoring are described in the Safety Monitoring Plan with the oversight of the DSMB.

3 STUDY DESIGN AND MANAGEMENT

3.1 Study Design Overview

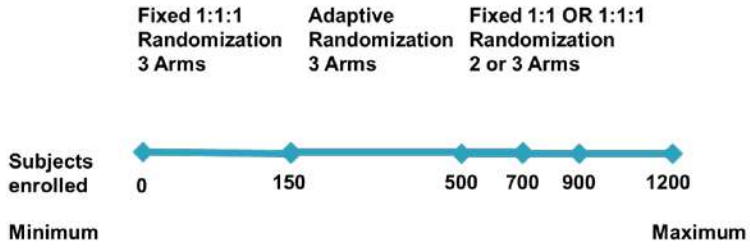
The MOST trial is a multi-arm, adaptive, single blinded, randomized controlled Phase 3 clinical trial conducted at up to 110 sites to determine whether argatroban and/or eptifibatide is superior to placebo in improving 90-day modified Rankin scores (mRS) in acute ischemic stroke (AIS) patients treated with IV rt-PA (0.9mg/kg) or TNK (0.25mg/kg) within three hours of symptom onset. We will also assess the safety of the combination therapies. A schematic of study activities is illustrated below.

Study Arms:

1. argatroban (100μg/kg bolus and a 12-hour infusion at 3μg/kg/min)
2. eptifibatide (135μg/kg bolus and a 2-hour infusion at 0.75μg/kg/min)
3. placebo

We will enroll a maximum of 1200 subjects. The first 150 subjects will be randomized in a 1:1:1 ratio to either argatroban, eptifibatide, or placebo. From the 150th to the 500th subject enrollment, response adaptive randomization (RAR) will favor the active arm showing the greatest benefit based on accrued data. After 500 subjects, one or both intervention arms may be carried forward for fixed randomization versus placebo. When N=500, one (or both) arm(s) may be stopped for futility if there is <20% chance of demonstrating benefit in either intervention (argatroban or eptifibatide) if the trial were to continue. Next, one (or both) arm(s) may be stopped for futility when N=700 or N=900, if there is <5% chance of demonstrating benefit in either intervention if the trial were to continue. One (or both) arm(s) may be stopped early for efficacy after 700 or 900 subjects if an arm has an expected success predictive probability of demonstrating superiority to control of ≥99%.

Figure 6 – MOST Trial Schematic



We will also assess the safety of argatroban and eptifibatide in combination with IV thrombolysis and ET. The National Data Management Center (NDMC) will generate periodic DSMB reports throughout the trial. The study's Independent Medical Safety Monitor (IMSM) will review all ICH cases throughout the trial and determine if a hemorrhage is symptomatic. We expect 30% of enrolled subjects will receive ET.[9,11] The endovascular safety monitor will review all ET cases for safety particular to ET.

3.2 Study Outcomes

3.2.1 Primary Outcomes

Primary Efficacy Outcome

The primary outcome measure for the MOST trial will be the 90-day mRS scores translated to patient-centered utilities.[45,46] The utility values assigned to each mRS score are shown below in Table 3 and the comparisons of the utility values to observed treatment effects in pivotal stroke trials are shown in Table 4.

Table 3 – Utility Scores for Each mRS Score

mRS Score	0	1	2	3	4	5	6
Rivero-Arias et al utility scores.[44]	10	8.7	7.3	6.0	2.8	-0.1	0
Hong & Saver utility scores[45]	10	9.5	7.9	6.7	3.5	0.1	0
MOST Trial utility scores	10	9.1	7.6	6.5	3.3	0	0

Table 4 – Effect Size Using Ordinal or Dichotomized and Utility-Weight Analysis of the Modified Rankin Scale

		Proportion of mRS		Utility-Weighted mRS			
Trial	Odds Ratio (95% CI)	% 0-1 (0-2) Control	% 0-1 (0-2) Treatment	Mean Utility Control	Mean Utility Treatment	Delta Utility Means	95% CI
NINDS TPA Trials^	1.7 (1.1-2.6)	26.6 (38.5)	42.0 (49.4)	5.01	5.90	0.89	0.31 – 1.49
MR CLEAN#	1.67 (1.21-2.30)	6.0 (19.1)	11.6 (32.6)	3.62	4.62	1.00	0.43 – 1.57
IMS 3^	1.08 (0.77-1.52)	8.9 (38.7)	12.8 (40.8)	5.05	5.41	0.36	-0.24 - 0.97
IST 3^	1.27 (1.10-1.47)	21.0 (35.0)	24.0 (37.0)	4.18	4.48	0.30	0.01 - 0.58
ECASS 3^	1.28 (1.00-1.65)	45.2 (61.6)	52.4 (66.5)	6.74	7.00	0.26	-0.18 - 0.70
^ dichotomized analysis; # ordinal analysis							

Primary Safety Outcome

The primary safety outcome will be the sICH rate defined as a type 2 parenchymal hemorrhage (PH2) or a remote parenchymal hemorrhage with neurological deterioration (≥4 point worsening in the NIHSS) within 36 hours of randomization.[47]

PH-2 refers to the following classification of ICH after ischemic stroke as proposed by Berger et al.[48]:

Table 5 – Classification of Hemorrhage

Type	Description
Hemorrhagic Infarct Type 1	Small petechiae along margin of the infarct
Hemorrhagic Infarct Type 2	More confluent petechiae within the infarcted area but without space-occupying effect
Parenchymal Hematoma Type 1	Hematoma in < 30% of the infarcted area with some slight space occupying effect
Parenchymal Hematoma Type 2	Dense hematoma > 30% total of the infarcted area with substantial space-occupying effect or any hemorrhagic area outside the infarcted area

3.2.2 Secondary Outcomes

Secondary Efficacy Outcomes

These will include the proportion of participants with NIHSS ≤2 at 24 hours (a strong predictor of long term outcome)[49]; change from baseline to 24-hour NIHSS[48]; proportion with 90-day mRS 0 or 1 and 0-2; 90-day ordinal analysis of the mRS[50]; 90-day EQ-5D[51]; the proportion of participants who have thrombectomy.

Secondary Safety Outcomes

These will include the proportion of participants with parenchymal hemorrhage types 1 (PH-1) and 2 (PH-2); any ICH on brain imaging within 36 hours of randomization; major hemorrhage (defined as requiring >2 units packed red blood cells) other than intracranial hemorrhage within 7 days; and, 90-day all-cause mortality.

3.2.3 Clinical Outcome Assessments – Blinded Determination of Day 90 mRS

Given the single blinded design, a blinded central assessor will determine the primary outcome in MOST. The clinical evaluation outcomes of interest for the MOST trial include the 24-hour NIHSS, the Day 30 and Day 90 mRS and the Day 90 EQ-5D.[51] All assessments will be conducted by certified study personnel. An individual who is blinded to treatment assignment should assess the 24-hour NIHSS. The Day 30 mRS evaluation will occur in-person or via telephone call with the subject and/or proxy (if the subject is unable). The telephone mRS has been shown to have good agreement with face-to-face mRS (weighted kappa 0.82, 95% CI 0.77-0.88).[52,53] The Day 90 mRS should be conducted by video recorded face-to-face interviews. If this is not possible, a recorded remote assessment by video or telephone will be used. The Day 30 and Day 90 mRS score will be assigned by local study personnel. A blinded central assessor will assign Day 90 mRS from video recordings performed by local personnel. The central mRS assignment will be used for the primary outcome assessment. Central mRS assessments will be recorded on videos which will be uploaded to a secure server where they will be checked, stored and distributed for independent scoring by expert raters, with committee discussion as appropriate.

3.3 Imaging Acquisition and Analysis

Imaging protocols: Heterogeneity of varying scanner capabilities and changing scanners at the participating sites is expected in multicenter imaging trials. Hence, absolute standardization is difficult. The imaging obtained will be standard of care and require that the imaging protocols at the enrolling sites are based on the Stroke Imaging Research (STIR) consortium recommendations, which have been designed to accommodate different makes of scanners.[54] This will increase the homogeneity of the imaging studies obtained at the different sites, and the central review will further ensure that the imaging biomarkers used in this study are comparable from site to site.

Imaging analysis: To protect confidentiality and prevent bias, all imaging data will be transmitted via a secure platform to the study neuroradiologist for central interpretation of: (1) Baseline non-contrast CT- Acute ischemia defined by ASPECTS score, (2) Baseline vascular imaging CTA/MRA- LVO (defined as intracranial ICA, MCA-M1 or basilar occlusion) and (3) 12-36 hour follow up noncontrast CT or MRI- safety monitoring for presence of hemorrhage and grading of hemorrhagic transformation.

Data collected include the baseline ASPECT score for all randomized subjects, the presence of LVO (yes or no) at baseline for patients undergoing ET, and presence of ICH, PH-1, and PH-2 on the 12-36 hour CT or MRI for all randomized subjects. These imaging data will be determined by the central imaging readers.

Of primary interest is the proportion of PH-2 ICH as this is used to define the primary safety outcome which will be adjusted for by the baseline ASPECTS score. At the end of the trial, the proportion of subjects with any ICH and the proportion who develop PH-1 and PH2 will be reported by treatment group. Other imaging data (e.g., M2 occlusions or collateral scores) will be noted and included in exploratory analyses separate from the aims of the present proposal.

3.4 Manual of Procedures

The Manual of Procedures will be a document containing detailed instructions on the recruitment process, study procedures and data collection. The document should be used by the clinical sites for day to day operations. Revisions to the manual will be made as necessary.

4 SELECTION AND ENROLLMENT OF SUBJECTS

4.1 Inclusion and Exclusion Criteria

Inclusion Criteria

1. Acute ischemic stroke patients
2. Treated with 0.9mg/kg IV rt-PA or 0.25mg/kg IV TNK within 3 hours of stroke onset or time last known well
3. Age ≥ 18
4. NIHSS score ≥ 6 prior to IV thrombolysis
5. Able to receive assigned study drug within 60 minutes but no later than 75 minutes of initiation of IV thrombolysis

Exclusion Criteria:

1. Known allergy or hypersensitivity to argatroban or eptifibatide
2. Previous stroke in the past 90 days
3. Previous intracranial hemorrhage, neoplasm, subarachnoid hemorrhage, or arterial venous malformation
4. Clinical presentation suggested a subarachnoid hemorrhage, even if initial CT scan was normal
5. Any surgery, or a biopsy of parenchymal organ in the past 30 days
6. Trauma with internal injuries or ulcerative wounds in the past 30 days
7. Severe head trauma in the past 90 days
8. Systolic blood pressure persistently >180mmHg post-IV thrombolysis despite antihypertensive intervention
9. Diastolic blood pressure persistently >105mmHg post-IV thrombolysis despite antihypertensive intervention
10. Serious systemic hemorrhage in the past 30 days
11. Known hereditary or acquired hemorrhagic diathesis, coagulation factor deficiency, hereditary fructose intolerance or oral anticoagulant therapy with INR >1.5
12. Positive urine or serum pregnancy test for women of child bearing potential
13. Glucose <50 or >400 mg/dl (<2.8mmol/L or >22.2 mmol/L)
14. Platelets <100,000/mm3
15. Hematocrit <25 %
16. Elevated pre-thrombolysis PTT above laboratory upper limit of normal
17. Creatinine > 4 mg/dl (>354 μmol/L)
18. Ongoing renal dialysis, regardless of creatinine
19. Received Low Molecular Weight heparins (such as Dalteparin, Enoxaparin, Tinzaparin) in full dose within the previous 24 hours
20. Abnormal PTT within 48 hours prior to randomization after receiving heparin or a direct thrombin inhibitor (such as bivalirudin, argatroban, dabigatran or lepirudin)
21. Received Factor Xa inhibitors (such as Fondaparinaux, apixaban or rivaroxaban) within the past 48 hours
22. Received glycoprotein IIb/IIIa inhibitors within the past 14 days
23. Pre-existing neurological or psychiatric disease which confounded the neurological or functional evaluations e.g., baseline modified Rankin score >3
24. Other serious, advanced, or terminal illness or any other condition that the investigator felt would pose a

significant hazard to the patient if rt-PA, TNK, eptifibatide or argatroban therapy was initiated
   a. Example: known cirrhosis or clinically significant hepatic disease
25. Current participation in another research drug treatment or interventional device trial - Subjects could not start another experimental agent until after 90 days
26. Informed consent from the patient or the legally authorized representative was not or could not be obtained
27. High density lesion consistent with hemorrhage of any degree
28. Large (more than 1/3 of the middle cerebral artery) regions of clear hypodensity on the baseline CT Scan. Sulcal effacement and/or loss of grey-white differentiation alone are not contraindications for treatment

4.2 Study Enrollment Procedures

At the enrolling center, thrombolysis patients will be screened by the treating Stroke Team physician based on the inclusion and exclusion criteria for the study. Subjects for the MOST trial will be recruited from all patients with suspected AIS admitted to participating hospitals. Given that time to treatment is critical for optimal outcomes, IV thrombolysis and ET will be planned and administered per usual care. Those not meeting eligibility criteria will continue to be treated per usual care and will not be enrolled in the trial. Those who meet inclusion and exclusion criteria will be approached for possible consent and enrollment. This must be done quickly as the patient should be consented and study drug started within 60 minutes of thrombolysis initiation without interfering with plans for ET if indicated. Study drug administration up to 75 minutes of thrombolysis is allowable. Telemedicine and other remote procedures may be used for informed consent per approval by the United States Central Institutional Review Board or the Canada Research Ethics Boards.

A consent process must be executed for all subjects entered in the trial. Obtunded patients are not automatically excluded from the study. If neither the subject nor the LAR (i.e., the individual legally empowered in the state where the consent is obtained) can provide consent, entry into the study will not proceed.

Once a subject has been deemed eligible and consent has been obtained, they may be randomized.
To randomize a subject, the qualified MOST study team member will log in to WebDCU™, then WebDCU™ evaluates the treatment arm distribution and generates a randomization number based on the randomization scheme. The randomization number corresponds to one of the study drug kits in inventory at the clinical site. A web-based central randomization system will be developed by the NDMC and implemented through the WebDCU™ MOST study website.

A screen failure log will be maintained by study site documenting reasons for trial ineligibility.

4.3 Participating Sites

Participating sites for MOST will be comprised of up to 110 enrolling sites in the United States and Canada.

5 STUDY INTERVENTIONS

5.1 Interventions, Administration and Duration

The study intervention will be one time administration of argatroban (100μg/kg bolus followed by 3μg/kg per minute for 12 hours) or eptifibatide (135μg/kg bolus followed by 0.75μg/kg/min infusion for two hours) or placebo. We anticipate subjects will be treated in the emergency department (ED) after presentation with AIS. Argatroban, eptifibatide, or placebo will be administered within 75 minutes of initiation of thrombolysis. All study medications must be stopped at 12 hours from the initial bolus of study medication regardless of interruptions to the infusion. Participants will be monitored closely in the intensive care unit (ICU) and monitored for neurological deterioration secondary to sICH. In-hospital clinical care, including indications for ET, will be per American Stroke Association (ASA) or Canadian Stroke Best Practice Recommendations (CSBPR) treatment guidelines.[55,56]

5.2 Handling of Study Interventions and Blinding

Commercially available argatroban and eptifibatide will be procured by the NIH StrokeNet Central Pharmacy. The Central Pharmacy will assemble the commercially available product into study drug kits and ship to Clinical Performing Sites in the United States for study use.

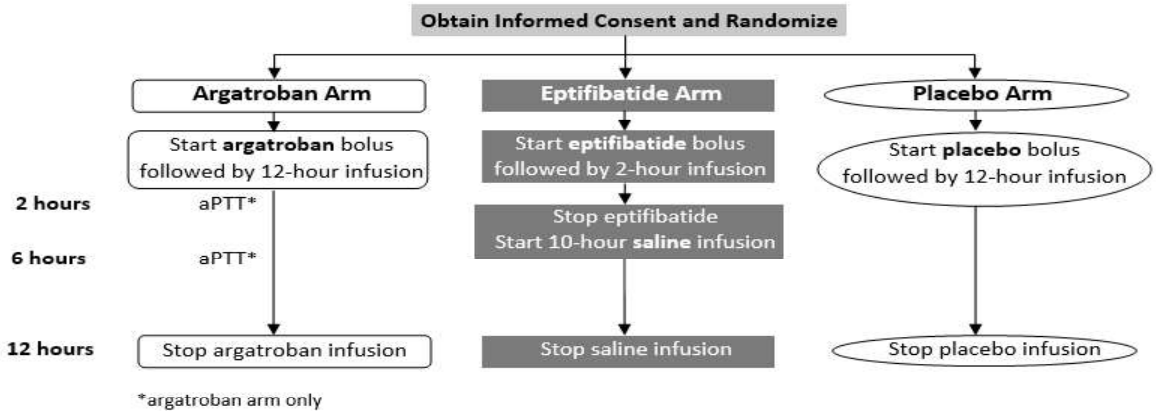
The Research Pharmacy at the University of Calgary will assemble commercially available argatroban and eptifibatide into study drug kits and ship to Clinical Performing Sites in Canada for study use.

5.2.1 Blinding

Argatroban and placebo subjects will initially receive a bolus followed by a 12-hour infusion. Eptifibatide arm subjects will receive a bolus followed by a 2-hour infusion. A 10-hour saline infusion will be administered after the 2-hour eptifibatide infusion to maintain the single blind. Investigators are unblinded to treatment arms, but subjects and LARs are blinded throughout the duration of the trial.

The two primary concerns of lack of blinding are: 1) knowledge of the treatment arm could bias endpoint assessments of safety (sICH) or efficacy (mRS at 90 days); and, 2) knowledge of an active treatment could influence the decision of investigators to proceed with ET in some participants, although we believe this is unlikely. Centralized blinded adjudication of sICH, and centralized blinded 90-day mRS assessments will mitigate the first concern, and requiring a clinical decision regarding a plan to proceed to ET prior to randomization will mitigate the second concern. A schematic of the blinding process for MOST is shown in Figure 7.

Figure 7 – Schematic of study treatment administration.



Once a patient is randomized, the study team member will retrieve the study drug kit from site inventory labeled with the randomization number assigned by WebDCU™. The study kit will contain saline placebo, argatroban or eptifibatide sufficient for administration to the maximum dosing weight of 100kg.

6 CLINICAL AND LABORATORY EVALUATIONS

6.1 Schedule of Events

Table 6 –Schedule of Events

Time	Baseline	2 hour (+/- 30 min) (after start of study drug)	6 hour (+/- 30 min)	24 hours (+/- 12 hrs)	Day 3/Discharge* (+/- 24hrs)	Day 30 (+/- 7 days)	Day 90 (+/- 14 days)
Inclusion Exclusion Criteria	X						
Subject Enrollment	X						
Informed Consent/ Randomization	X						
History & Physical[#]	X						
NIH Stroke Scale	X			X			
Modified Rankin Score	X					X	X
EQ-5D							X
CT/MRI scan (SOC[#])	X			X			
CTA/MRA (if SOC)	X						
CBC with platelets[#]	X						
Glucose, electrolytes, BUN/creatinine, PT[#]	X						
aPTT	X[#]	X[$]	X[$]				
Dosing Titration[$]∞		X	X				
Adverse events	X	X	X	X	X	X^	X^
End of Study							X

[#]Standard of care *whichever comes first ^serious AEs only [$]argatroban arm only ∞as needed based on aPTT titration protocol

6.2 Timing of Evaluations

6.2.1 Pre-Intervention Evaluations

These evaluations occur prior to the subject receiving any study interventions.

Screening

A history, physical examination, and NIH stroke scale will be performed by the Stroke Team physician, and intravenous thrombolysis will be started per usual care in eligible patients. Thrombolysis is to be started as soon as possible in the ED, and delays in initiating therapy are to be avoided. For patients eligible for ET, rapid transport to ET should be initiated and facilitated prior to engaging in discussions regarding the study.

Entry/Baseline

If thrombolysis is started within 3 hours from symptom onset, the patient will be evaluated for eligibility for the trial. Informed consent will be obtained from the patient (if able) or LAR. A baseline modified Rankin score will be recorded to indicate the subject's functional status prior to the qualifying stroke (pre-event). Baseline usual care imaging and lab data points that will be collected include the CT/CTA findings, complete blood count results, glucose and electrolyte panel with BUN/creatinine, coagulation parameters (including pre-thrombolysis PT/PTT/INR) and urine pregnancy tests in women of child-bearing age.

6.2.2 On-Study Evaluations

The NIH stroke scale score will be performed on every subject immediately prior to initiation of intravenous thrombolysis therapy and at 24 hours post initiation of study drug. The NIHSS will be done by an NIHSS certified investigator. A non-contrast head CT or magnetic resonance imaging (MRI) of the brain (per usual care) will be performed at baseline and within 36 hours of randomization. An aPTT will be assessed at 2 hours and 6 hours, according to the WebDCU™ generated instructions, in argatroban arm subjects. All adverse events will be reported from randomization through Day 3 or Discharge, whichever comes first. After Day 3/Discharge, only serious AEs and safety outcomes will be reported. At Day 30 and Day 90, the modified Rankin score will be collected. The EQ-5D will be collected at Day 90.

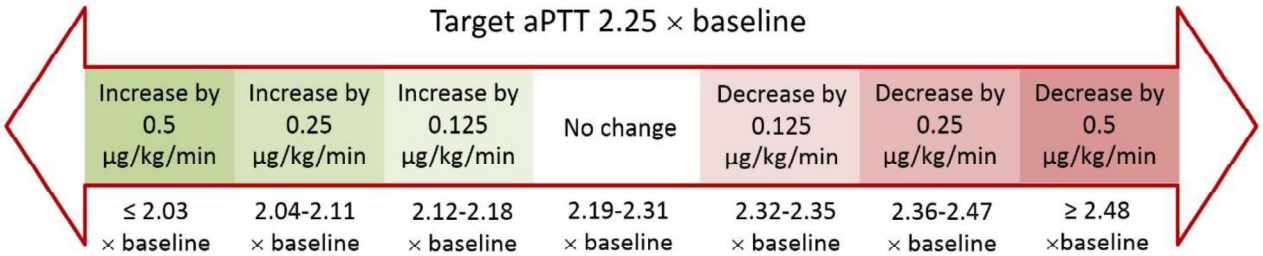
6.2.3 Intervention Discontinuation Evaluations and Premature Withdrawals

Per standard of care after treatment with thrombolysis, the infusion should be discontinued and a repeat head CT performed if the participant develops sudden neurological worsening or severe headache during treatment. If ICH is detected on CT, reversal agents including prothrombin complex concentrate (PCC), cryoprecipitate, platelets and fresh frozen plasma should be administered. A neurosurgery consultation should be obtained. Guidance for management of ICH is provided in section 8.1.

6.2.4 Titration of Infusions

Argatroban requires titration to target aPTT at 2 and 6 hours after initiation, according to the WebDCU™ generated instructions. For argatroban arm subjects, clinical personnel will titrate the study drug infusion per protocol.

Figure 8 – Protocol for aPTT Titration



These assume an infusion dose of 3µg/kg/min. As such, if a 100kg subject has an aPTT ≤2.03 x baseline after a 2-hour infusion, the infusion from 2-12 hours will be increased from 3µg/kg/min to 3.5µg/kg/min, or 18 mL/hr to 21 mL/hr, of 1mg/mL argatroban.

6.2.5 Post-Intervention Evaluations

Per standard of care, the subject will be admitted to the ICU and neurological assessments performed hourly for 24 hours. Clinical care and use of ET will be per American Stroke Association (ASA) or Canadian Stroke Best Practice Recommendations (CSBPR) guidelines.[55,57,56]

6.2.6 Final Evaluations and Unscheduled Visits

In-hospital assessments will occur daily for 3 days or until Discharge, whichever comes first. The Day-30 visit will be completed over the phone or in-person. The final assessment will occur at Day 90. Every effort should be made to conduct this visit in-person. The mRS interview should be video recorded.

7 CONCOMITANT TREATMENTS

Concomitant use of antiplatelet or anticoagulant medications is prohibited in the first 24 hours after initiation of thrombolysis unless a safety scan shows it is safe to administer in an effort to avoid common deviations. Thereafter, antiplatelet agents may be initiated per usual care. Anticoagulant medications are discouraged per ASA and CSBPR guidelines,[55,58] except for venothromboembolism prophylaxis. In the event that systemic anticoagulation is thought clinically necessary, the route, frequency, dose and duration must be documented. Endovascular thrombectomy may occur per usual care. For ET patients, heparin may be used for line flushes only but no systemic antithrombotic agents are permitted.

8 CRITERIA FOR INTERVENTION DISCONTINUATION

The most likely reasons for discontinuing the intervention after enrollment are acute neurological deterioration possibly secondary to sICH during the infusion, and refractory hypertension (blood pressure cannot be maintained below 180/105mmHg despite a continuous infusion medication). In both cases, the rt-PA and/or study drug are to be stopped immediately. The subject should continue to be followed and data collected per the study protocol.

8.1 Management of Intracranial Hemorrhage

Reversal of Intracranial Hemorrhage

Reversal of intracranial hemorrhage is key for all treatment arms since IV thrombolysis will be administered to all enrolled subjects. However, the overall goal of stopping bleeding remains the same and thus the reversal approach is similar for all three treatment arms (see below).

Management of intracranial hemorrhage

Intracranial hemorrhage should be suspected if there is any acute neurological deterioration (new headache, acute hypertension, seizure, or nausea and vomiting) or acute increase in BP.

If ICH is suspected:

- Discontinue rt-PA and/or study drug infusion until ICH is ruled out
- Immediately perform CT scan
- Draw coagulation tests that may include INR, PT, aPTT, platelet count, fibrinogen, thromboelastography (TEG) and type and screen
- If ICH is not present, re-start the rt-PA and/or study drug infusion at the discretion of the investigator

If ICH present:

- Administer thrombolysis reversal agents per local protocol
- Consider emergent neurosurgical consultation
- Discuss with participant's family or next-of-kin

For patients who received eptifibatide:

- Consider DDAVP infusion (0.3mcg/kg IV x 1at the discretion of investigator); and/or
- Consider a transfusion of platelet concentrates (based on absolute low platelet count <100k or reduced maximum amplitude [MA] value on TEG) per local protocol or Cryoprecipitate (if prolonged K-value on TEG) in case of major or life-threatening bleeding or urgent need for normalization of platelet function in case of surgery

9 ADVERSE EVENT ASCERTAINMENT AND REPORTING

9.1 Definition of Safety Outcomes

The following are safety outcomes of the study:

- sICH within 36 hours from randomization
- Proportion of participants with parenchymal hemorrhage types 1 (PH-1) and 2 (PH-2) within 36 hours of randomization
- Any ICH on brain imaging within 36 hours of randomization
- Major hemorrhage (requiring >2 units of packed red cells) other than intracranial hemorrhage within seven days of randomization
- All-cause mortality within 90 days of randomization

Symptomatic Intracerebral Hemorrhage: A type 2 parenchymal hemorrhage (PH-2) or remote parenchymal hemorrhage associated with a four or more point increase in the NIHSS score from baseline to subsequent CT scan at the time of worsening, per SITS-MOST definition.[47] Hemorrhage classification will be based on central neuroradiologist review and the final judgment regarding whether the hemorrhage is symptomatic will be made by the independent safety monitor.

9.2 Reporting of Adverse Events

All adverse events (AEs), all Serious AEs, and all safety outcomes will be reported from randomization through Day 3 or Discharge, whichever comes first. After Day 3/Discharge, all serious AEs and all safety outcomes will be reported. For each reportable AE, an AE case report form (CRF) will be submitted in WebDCU™ capturing the details of the event including date of onset, severity, duration, and relationship to the treatment. For serious AEs, additional information, including narrative summaries will be submitted.

Non-serious events are reported in WebDCU™ within 5 days of the site PI and primary study coordinator's awareness of the event, and serious adverse events are reported within 24 hours of the site PI and primary study coordinator's awareness of the event. Sites are responsible for updating AE reports with new information as it becomes available (e.g., date of resolution, action taken). All serious AEs must be followed for the duration of the study follow-up or until resolution, whichever comes first. Upon completion of the study protocol by the subject, premature withdrawal from the study by the subject, or the subject's death, all information regarding each AE must be completed, if not done earlier.

If an event is determined to be unexpected (not previously observed), related to trial participation (study drug) and meets FDA and/or Health Canada criteria as a serious event, an expedited safety report will be submitted to the FDA in accordance with CFR 312.32 and to Health Canada in accordance with C.05.014.

9.2.1 Definition of Relatedness

Details will be provided in the MOP. For each Adverse Event, the relationship to the study treatment (relatedness) must be recorded as one of the choices on the following scale:

- Not Related
- Unlikely

- Reasonable possibility
- Definitely

10 STATISTICAL CONSIDERATIONS

This section is an overview of the statistical considerations. Complete details can be found in the Statistical Analysis Plan (SAP). The general overview of the design is provided in section 3.1.

10.1 Sample Size and Accrual

The minimum clinically significant difference was defined as 0.4 points on the utility scale primary outcome, which corresponds to ~45% of the effect for IV rt-PA over placebo in the NINDS study (Table 4).[59] With a maximum N=1200, this design provides at least 80% power to detect a treatment effect when the overall true utility benefit is 0.4 for one active arm in non-ET subjects (Table 7). If both treatment arms are equally effective at 0.4 utility above the control arm, power is 89%. The properties of the study design have been investigated through a series of simulations that assumed 30% of patients receive ET, and the treatment effect for ET patients is 50% of the effect in non-ET patients, corresponding to 25% of the treatment effect of rt-PA over placebo. The impact of ET on treatment effect is unknown but we assumed the worst scenario, i.e., marked attenuation of effect. Table 7 shows power when only one treatment arm is effective. Power increases if <30% of patients receive ET.

Table 7 – Power for Varying Treatment Effects if only One Arm is Truly Effective

	Utility Values Above rt-PA Alone		
	Scenario 1	Scenario 2	Scenario 3
Assumed True Effect Overall (70% non-ET and 30% ET subjects)	0.26	0.34	0.425
Assumed True Effect for non-ET subjects	0.30	0.40	0.50
Assumed True Effect for ET subjects (50% of non-ET effect)	0.15	0.20	0.25
Pr{Effective arm wins}	0.52	0.80	0.95
Pr{Other arm wins}	0.011	0.008	0.014

Numbers in gray are the utilities (difference in the expected treatment minus control). Numbers in white are the power under 3 different scenarios (determined by simulation).

Given the non-inferiority of TNK and similarity in efficacy and safety between TNK and rt-PA,[41] we do not anticipate treatment effect to be different between subjects treated with rt-PA versus TNK as standard of care thrombolysis. Further, our simulations included treatment effect as low as 0.255 utility points as detailed in our SAP. Thus, we have reasonable power even if there is some attenuation of treatment effect by thrombolysis approach.

10.2 Data Analysis

10.2.1 Primary Efficacy Analysis

The primary analysis will be conducted on the intent-to-treat (ITT) analysis population, defined as all subjects randomized regardless of the treatment actually received. The primary analysis will be a two-sample comparison of each active arm compared to placebo mRS utility scores adjusting for severity (baseline NIHSS). If both active arms reach the end of the trial, they will each be compared to control independently. The final analysis is Bayesian and includes a flexible normal dynamic linear model (NDLM) to account for different expected outcomes as a function of initial NIHSS. This is a flexible spline-like model that will capture that the average weighted mRS score in the control group as a (possibly non-linear) function of initial NIHSS. Meanwhile for a given treatment d, θ_d, is the difference in the expected utility for the active treatment minus control. For each active arm, the hypothesis test is

$$H_0: \theta_d \leq 0$$
$$H_A: \theta_d > 0$$

The treatment effect θ_d is given a vague prior, θ ~ N(0,2.5^2). Particularly, the prior probability that a drug is beneficial is the same as the prior probability that it is harmful. If there is a high posterior probability that the treatment effect θ_d is positive, the treatment is declared efficacious. The posterior probability is conditional on the final results for all enrolled subjects. If two active arms remained in the trial all the way to the end, this posterior probability is computed for each, and potentially both arms can be declared to be successful.

The primary output of the final analysis is the posterior probability that $\theta_d > 0$, for any d's that remain in the trial. If this probability is at least 0.985, the trial is considered a success. The threshold for defining significance is chosen so that the Type I error is no larger than 0.025. Criteria for success (critical value for posterior probability of a positive benefit) was inflated from 0.975 to 0.985 to account for the two study drugs and the repeated interim looks (e.g. the trial can be stopped when data look favorable enough that success is likely).

10.2.2 Interim Analyses

The interim evaluations of the primary efficacy outcome data are described in the Statistical Analysis Plan. A brief description is provided under section 3.1.

The primary safety outcome (sICH) proportion for each treatment group (overall and by ET/non-ET subgroups and thrombolysis approach, i.e., TNK versus rt-PA) will be compared to the expected rate of 3%. The probability that the safety outcomes in an intervention arm exceeds the expected rate will be provided in each DSMB report. Stopping rules are non-binding and are to be used as a guideline by the DSMB and safety monitor in conjunction with other relevant data and careful judgement.

10.2.3 Secondary and Exploratory Analyses

The primary outcome of mRS utility values will be reanalyzed in a multiple linear regression adjusting for baseline NIHSS, age, time to IV thrombolysis, thrombolytic agent (rt-PA or TNK), location of LVO and time from onset to groin puncture in subjects undergoing ET. All secondary analyses will be tested at a significance level of two-sided α=0.05. For binary outcomes each active treatment arm will be compared to control in a chi-squared test or fisher's exact test. For ordinal outcomes each active treatment arm will be compared to control in a Wilcoxon Rank-Sum test and ordinal logistic regression. For continuous outcomes, each active treatment arm will be compared to control in a two-sample t-test. Kaplan Meier curves and log rank tests will be used to compare time to 90-day mortality by treatment group.

Exploratory analyses of the secondary outcomes will use either logistic regression (for binary outcomes), ordinal logistic regression (for categorical outcomes) or multiple linear regression (for continuous outcomes) to compare treatment groups after adjusting for age, baseline NIHSS, time to IV thrombolysis, thrombolytic agent, location of LVO and time to groin puncture in ET subjects.

10.2.4 Subgroup Analysis: Gender, Race, Ethnicity

Although we do not anticipate differential treatment effects based on gender, race, or ethnicity, our analyses will explore clinically important differences due to race/ethnicity. The mRS utility values will be reanalyzed in a multiple linear regression adjusting for baseline NIHSS including indicator variables of sex, racial group, and ethnic group and interaction terms with treatment group.

11 STUDY MONITORING

11.1 Data Monitoring

Monitoring for this study will be performed by the NDMC centrally for the US and Canada. The NDMC will perform on site and remote monitoring for US sites. The University of Calgary in partnership with NDMC will perform on site and remote monitoring for Canadian sites. Per the study's monitoring plan, monitoring will include a combination of on-site monitoring (to verify data entered into the WebDCU™ database against source documents and query inaccuracies between the source documents and WebDCU™ database), remote monitoring (source document verification, including verification of written consent, may be performed remotely

by reviewing source documents that have been uploaded into WebDCU™ or via remote access to electronic medical records), and central monitoring (using web-based data validation rules, data manager review of entered data, statistical analysis, and on-going review of site metrics).

In an effort to review informed consent forms in a timely manner, enrolling sites that are permitted to do so, will upload a PDF of the signed informed consent form, into the password protected clinical trial management system, WebDCU™. The PDF file will be linked to the subject ID but will be stored on a secure server separate from the study's CRF data. The secure server on which these files are stored is not backed up to prevent copies of files containing Individually identifiable health information from being copied and stored on non-NDMC back up servers. The files on these servers can only be accessed by designated study personnel upon entry of a second password. NDMC staff will remotely monitor the informed consent forms and issues identified will be relayed to the clinical site for corrective and preventative action. After remote monitoring is complete, the PDF file containing the informed consent form will be permanently deleted from the secure server. If a subject must be re-consented, the process will repeat itself.

For the Canadian sites, the University of Calgary will manage informed consent verification according to Canadian regulations.

Further details of clinical site monitoring are documented in the study's Monitoring Plan. The Monitoring Plan describes in detail who will conduct the monitoring, at what frequency monitoring will be done, at what level of detail monitoring will be performed, and the distribution of monitoring reports.

11.2 Data Management

Data management will be handled by the StrokeNet NDMC which is housed in the Data Coordination Unit (DCU) in the Department of Public Health Sciences at the Medical University of South Carolina (MUSC) which is described below. All activities will be conducted in coordination with the study PIs, the StrokeNet National Coordinating Center, and the clinical sites (StrokeNet and non-StrokeNet). In addition to the study database, the NDMC will provide the clinical site staff access (via password) to a standard set of web-enabled tools, including subject visit calendar, subject accrual status, case report form completion status, and outstanding data queries pertaining to their respective clinical sites.

11.3 Quality Assurance and Quality Control

Data quality assurance processes at the NDMC include:

- Logic and rule checks built into the study database;
- Real-time, central monitoring by the data managers and statistical programmers at the NDMC; and
- Remote and on-site risk-based source verification monitoring by site monitors and data managers at the NDMC.

Quality control (QC) procedures will be implemented beginning with the data entry system, and data QC checks that will be run on the database will be generated. Any missing data or data anomalies will be communicated to the site(s) for clarification/resolution.

Following written procedures as detailed in the monitoring plan, the monitors will verify that the clinical trial is conducted and data are generated, documented (recorded), and reported in compliance with the protocol, GCP, and the applicable regulatory requirements (e.g., Good Laboratory Practices (GLP), Good Manufacturing Practices (GMP).

The investigational site will provide direct access to all trial-related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

12 HUMAN SUBJECTS

12.1 Institutional Review Board (IRB)/Research Ethics Board (REB) and Informed Consent

United States

The University of Cincinnati Institutional Review Board will serve as the National Central Institutional Review Board for participating sites in the United States. The Central Institutional Review Board (CIRB) for multicenter protocols is the single IRB of record for those sites. It has regulatory responsibility for assuring the protection of the rights and welfare of research participants in accordance with Standard Operating Procedure ADM 12; Central Institutional Review Board Reporting. The National Institute of Neurological Disorders and Stroke (NINDS) selected the University of Cincinnati Institutional Review Board (IRB) to serve as the CIRB for the NIH StrokeNet (StrokeNet) for sites in the United States.

Canada

Each site in Canada will utilize a REB to review research involving humans to ensure the protocol is conducted according to the Canada Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans (2018) and international standards for Good Clinical Practice. Applicable government regulations and site institution research policies and procedures will also be followed. The REB will be responsible for reviewing the protocol and the informed consent documentation.

Article 3.8 in the TCPS-2 outlines when REBs may grant deferral of consent/waiver of consent.[60] Canadian sites may seek deferral of consent where possible.

12.2 Recruitment of Minorities and Women

The individual age, race, and gender of each participant will be transferred to the NINDS at fixed intervals per the NINDS "Standard Operating Procedure of Email Recruitment Enrollment". The proposed study will take place in communities where there are diverse populations.

Inclusion of Women

All eligible patients of both genders will be approached to participate in the MOST trial. Only pregnant females are systematically excluded by this protocol because of potential risks to the unborn fetus. While there have been historical disparities in the proportion of women enrolled in stroke trials, our Phase 2 trials suggest we should adequately enroll equal numbers of both genders into MOST:

Table 8 - Inclusion of Women in the Phase 2 Trials

Trial	Women, N (%)	Men, N (%)
ARTTS	36 (55)	29 (45)
ARTSS-2	40 (44)	50 (56)
CLEAR	39 (42)	55 (58)
CLEAR-ER	60 (48)	66 (52)
CLEAR-FDR	14 (52)	13 (48)

Further, upper age limits in other stroke clinical trials may contribute to a bias towards enrolling more men than women since women tend to be older than men at the time of a first stroke. Thus, the absence of an upper age limit in MOST will allow us to avoid this bias by enrolling all eligible patients aged 18 or older. Overall, we expect to enroll approximately equal proportions of men and women. Further, while we do not anticipate differential treatment effects based on gender, our analyses will explore clinically important differences due to gender.

Inclusion of Minorities

All eligible patients of all races and ethnic groups will be approached to participate in MOST. In our analysis of discharged ischemic stroke patients in the Medicare Provider and Analysis Review (MEDPAR) administrative dataset, StrokeNet hospitals tended to have a lower proportion of white patients compared with non-StrokeNet hospitals (76.5% versus 82.8%) (Adeoye International Stroke Conference 2015). Thus, the stroke patients that will be available within StrokeNet for MOST are up to 23.5% non-white/minority. Particularly, StrokeNet centers such as University of Texas, Houston (city population is 24% Black or African American and 44%

Hispanic or Latino), Emory University (city population is 54% Black or African American), University of Miami (city population is 66% Hispanic or Latino), among others should facilitate adequate representation of minorities enrolled. In addition, we have solicited participation from non-StrokeNet sites with diverse patient populations.

Despite the availability of representative populations, available literature suggests barriers persist in the participation of minorities in clinical trials. Thus, we will take the following additional steps to ensure representative enrollment of minorities:

1. Emphasize the goal of recruiting a diverse population of stroke patients to participating sites.
2. Make clear to potential participants that patients of all races and ethnicities are being recruited.
3. Make consent form documents available in Spanish.
4. Review blinded aggregate information on demographic characteristics of enrolled patients after each 100 enrollments, and consider refocusing recruitment efforts towards sites with larger proportions of minorities as warranted.

Finally, while we do not anticipate differential treatment effects based on race or ethnicity, our analyses will explore clinically important differences due to race/ethnicity.

12.3 Subject Confidentiality

Participant confidentiality is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their agents. Therefore, the study protocol, documentation, data, and other information generated will be held in strict confidence. No information about the study or data will be released to any unauthorized third party without prior written approval of the sponsor.

The study monitor, other authorized representatives of the sponsor, Food and Drug Administration (FDA), Health Canada, and representatives of the NCC, NDMC and CIRB/REBs may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for the duration specified by the StrokeNet Standard Operating Procedure (SOP) or longer as dictated by the CIRB/REBs and local country regulations.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored for the duration of the study and analysis at the NDMC. The study data entry and

study management systems used by clinical sites and by the NDMC research staff will be secured and password protected. At the end of the study, all study databases will be de-identified and a Public Use Dataset (PUDS) will be archived with NINDS.

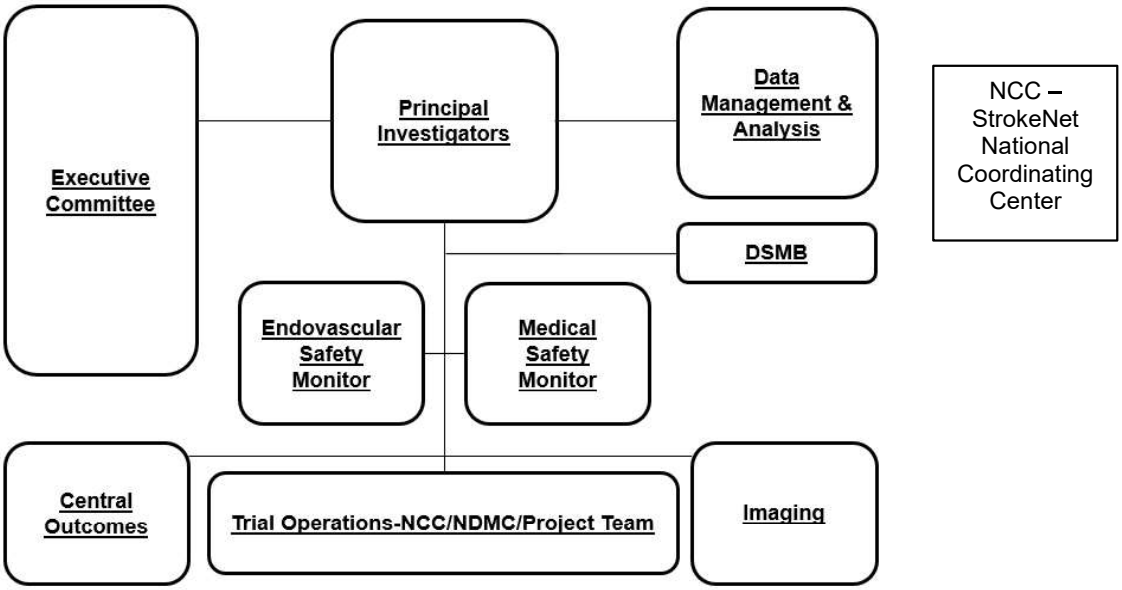
12.4 Study Modification/Discontinuation

The study may be modified or discontinued at any time by the DSMB, CIRB/REBs, the NINDS, the sponsor, the OHRP, the FDA, Health Canada, or other government agencies as part of their duties to ensure that research subjects are protected.

13 TRIAL ADMINISTRATIVE STRUCTURE

13.1

Figure 9 – MOST Organizational Chart



13.2 StrokeNet National Coordinating Center and National Data Management Center

The clinical coordination of the MOST Trial operations will be centralized through the following:

NIH StrokeNet National Coordinating Center (NCC)
University of Cincinnati
260 Stetson Street, Suite 2300
Cincinnati, Ohio 45267-0525

A Project Manager at the NCC will be assigned to coordinate the following study oversight: trial communication, required training activities, site assessment and or initiation visits, collection of trial related regulatory documents, recruitment performance tracking and performance analysis.

The data coordination and analysis of the MOST Trial will be centralized through the following:

NIH StrokeNet National Data Management Center (NDMC)
Medical University of South Carolina
135 Cannon Street
Charleston, SC 29425-8350

The study data will be managed (including data queries) by the NDMC using the WebDCU™ system. This user-friendly web-based database system, developed and validated by the NDMC, 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.

13.3 Data Safety and Monitoring Board (DSMB)

MOST will have an independent Data Safety Monitoring Board (DSMB) appointed by the NIH to oversee study safety. We do not expect safety concerns in non-ET patients based on our Phase 2 trials. Given the ARTSS-IA data, we do not expect combination therapy in the setting of ET will prove to be unsafe early in the conduct of the trial. The DSMB may stop an arm at any point for safety concerns. The DSMB will specify the content and frequency of data reports to be generated by the NDMC.

13.4 Independent Medical Safety Monitor

An Independent Medical Safety Monitor will review all SAE reports submitted by the clinical sites throughout the trial and will determine if reported intracranial hemorrhages are symptomatic. He/she also will be responsible for ensuring good clinical practice and to identify safety concerns quickly. The IMSM may suggest protocol modifications to prevent the occurrence of particular AEs, e.g., modifying the protocol to require frequent measurement of laboratory values predictive of the event or to improve expeditious identification of SAEs. To minimize bias, he/she will evaluate SAEs blinded to treatment assignment, unless the DSMB approves partial or complete unblinding. In the event of unexpected SAEs or an unduly high rate of SAEs, the IMSM will promptly contact the Lead PI and the NINDS Program Official who will notify the DSMB Chair.

13.5 Endovascular Safety Monitor

The endovascular safety monitor will review all SAE reports submitted by clinical sites that involve ET cases to assess safety particular to ET. He/she will ensure there are no safety issues particular to ET, including as performed by individual interventionists or sites. The endovascular safety monitor may suggest protocol modifications to prevent the occurrence of particular AEs, e.g., modifying the protocol to require frequent measurement of laboratory values predictive of the event or to improve expeditious identification of SAEs. To minimize bias, he/she will evaluate SAEs blinded to treatment assignment, unless the DSMB approves partial or complete unblinding. In the event of unexpected SAEs or an unduly high rate of SAEs particular to ET subjects, the endovascular safety monitor will promptly contact the Lead PI and the NINDS Program Official who will notify the DSMB Chair.

13.6 Executive Committee

The Executive Committee will provide overall clinical guidance and leadership for the execution of the MOST Trial. This committee will provide a means of partnership between the investigators, the NINDS, and the sponsors. The Executive committee, composed of experts in emergency medicine, vascular neurology, endovascular therapy and neuroimaging, and biostatistics will provide the overall scientific guidance for the study. The committee will meet at least quarterly by phone (1 hour) for the full duration of the study. Responsibilities include oversight of the overall conduct of the study with regard to protocol compliance and modifications/amendments, study progress, and problem-solving. The Lead PI will chair the executive committee.

13.7 National Institute of Neurological Disorders and Stroke (NINDS)

The NINDS will provide funding for all aspects of this trial via the NIH StrokeNet. An identified Program Official will be responsible for oversight of this trial.

14 PUBLICATION OF RESEARCH FINDINGS

Publication of the results of this trial will be governed by the policies and procedures developed by the Executive Committee. Any presentation, abstract, or manuscript will be made available for review by the sponsor and the NINDS prior to submission.

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