MISTIE III
A phase III, randomized, open-label, 500-subject clinical trial of minimally invasive surgery plus rt-PA in the treatment of intracerebral hemorrhage.

MTI-M3
Mechanisms of Tissue Injury in MISTIE III
Rebleeding and inflammation: predicting risk of excessive bleeding in minimally invasive surgery and inflammatory marker evaluation

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AGREEMENT ON THE PROTOCOL

Trial ID: **MISTIE III**
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IND #: 8523

The Principal Investigator (hereafter referred to as Investigator) and The Johns Hopkins Medical Institutions (hereafter referred to as JHMI) agree to conduct the trial as outlined in this protocol with reference to national/local/international regulations and in accordance with current *Good Clinical Practice* (GCP) and *International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use* (ICH).

Any modification to the protocol must be agreed upon by both the Investigator and JHMI and documented in writing. By written agreement to this protocol, the Investigator agrees to allow direct access to all documentation, including source data, to authorized individuals representing JHMI (including monitoring staff and auditors), to Institutional Review Boards (IRB) and/or to regulatory authorities.

Signature: _____ Date: _____

Name Printed: _____

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SYNOPSIS

Study Title

MISTIE III. A phase III, randomized, case-controlled, open-label, 500-subject clinical trial of minimally invasive surgery plus rt-PA in the treatment of intracerebral hemorrhage.

Objectives

Primary Objectives:

Efficacy: Demonstrate that minimally invasive surgery (MIS) plus recombinant tissue plasminogen activator (rt-PA) for three days improves functional outcome by a 12% increase in the modified Rankin Scale (mRS) score 0-3 compared to medically treated subjects assessed at 180 days.

Safety: Demonstrate that early use of MIS+rt-PA for three days is safe for the treatment of ICH relative to rates of mortality, rebleeding, and infection in the medically treated subject at 30 days.

Secondary Objective: Demonstrate that the end of treatment volume and percent of ICH reduction from MIS+rt-PA is related to improved functional outcome, as compared to medically treated subjects.

Design and Outcomes

This study is a phase III, randomized, open-label, multicenter evaluation of MIS and ICH lysis with rt-PA versus medical care. The study (n=500) will evaluate the efficacy and safety of MIS plus 1 mg of rt-PA administered every eight hours for up to nine doses as compared to subjects treated with conventional medical management. Endpoint assessment will be performed by blinded investigators at the University of Glasgow.

Subjects enrolling in this study may also consent to participate in an ancillary study titled *Mechanisms of Tissue Injury in MISTIE III*. This ancillary study offers a tremendous opportunity to leverage clinical trial data to bring novel insights from ICH pathophysiology into the clinical realm, using neuroimaging, genetic and inflammatory markers of disease to provide clinicians with powerful new tools to guide surgical therapy and develop new therapeutic targets. See Appendix 8 for the ancillary study protocol.

Interventions and Duration

The study is proposed to require five years. All subjects will be followed daily for six days post randomization. Subjects randomized to receive the surgical intervention will

undergo aspiration of clot followed by up to nine drug administrations. All subjects will be required to attend follow-up clinic visits at 30, 180, and 365 days after onset of ICH. A telephone follow-up will occur at 90 and 270 days.

Sample Size and Population

The study population will include 500 subjects, adaptively randomized 1:1 across approximately 90 to 100 study centers, with supratentorial ICH without suspected underlying structural etiology (tumor, vascular malformation or aneurysm). Subjects will be identified and recruited through the Emergency Department, clinical stroke service, and direct admissions to the Neurocritical Care Unit at each study center. See section 9.1.1. Randomization below for a more detailed description of the randomization procedure.

1 STUDY OBJECTIVES

1.1 Primary Objective (Efficacy)

Demonstrate that minimally invasive surgery (MIS) plus recombinant tissue plasminogen activator (rt-PA) for three days improves functional outcome by a 12% increase in the modified Rankin Scale (mRS) score 0-3 compared to medically treated subjects at 180 days.

1.2 Primary Objective (Safety)

Demonstrate that early use of MIS+rt-PA for three days is safe for the treatment of ICH relative to rates of mortality, rebleeding, and infection in the medically treated subject at 30 days.

1.3 Secondary Objective:

Demonstrate that the end of treatment volume and percent of ICH reduction from MIS+rt-PA is related to improved functional outcome, as compared to medically treated subjects.

2 BACKGROUND

2.1 Rationale

Scope of Problem: Brain hemorrhage is a worldwide problem without strategies for treatment or prevention.[1] Its incidence is persistent and its prevalence keeps increasing with an aging population.[2-4] The high burden of disease is well-established with a 30-day mortality of approximately 40%.[2] Substantial health disparities exist, with an increased incidence for Asians, African-Americans, Hispanics, those without access to blood pressure (BP) management, and the aged.[5]

ICH affects a young and productive population with morbidities that produce disastrous economic and social consequences,[3,6,7] and functional impairment produces an intolerable degree of dependency.[8] When hemorrhages of all sizes are considered, only 10%-25% return to functional independence, and functional performance is likely to be much worse (≤10%) when initial hematoma volumes are greater than 20-30 mL.[3,9,10]

We deliver care in a default position, where we provide complex brain monitoring and ICU support without evidence of benefit or emphasis on mitigation. The state of care for ICH is similar to care for ischemic stroke three decades ago—care is frequently not rendered. When rendered, it is palliative and supportive of cardiorespiratory function rather than reversing the brain injury. The absence of an evidence-validated treatment is associated with high cost, variability in care, uncertainty in decision-making, unacceptable mortality rates, and long-term functional dependency requiring skilled nursing.[11-13] The ICU stay for a patient with a moderately sized ICH (>30 mL) averages a month, requiring prolonged ventilation, nutrition support, and acute cardiovascular and infection treatments.[14] Health professionals make widely variable treatment, level-of-care, and prognostic decisions without trial evidence[15,16] or consideration of long-term survivor benefit/burden.[15-18]

Similarly, routine ***surgical*** decision-making (patient selection, procedure selection, & timing) is done without an evidence-based model;[11] yet surgery is often offered as a last life-saving resort.[11,19-21] A small number of clinical trials have produced results that greatly inform patient selection but showed no benefit for early craniotomy, ultra-early craniotomy, ultra-early treatment of bleeding, or early neuroprotection.[22] A practical approach—craniotomy to remove clots in all patients, from all locations, regardless of stability—is neither unequivocally accepted[18] nor fully evidence-based.[11] Craniotomy being only slightly better than medical management leaves strong community equipoise around a good body of evidence regarding volume-reduction therapy[20,23,24] and, more specifically, the MISTIE image-guided approach.

ICH is unlike stroke, in that the mechanism of injury, the requirements of care, and the likely pathway to a primary treatment are different.[22] The current default position falls short of mitigating the primary injury pathway.[22] An effective treatment is urgently needed that reduces impairment and increases functional independence in the home.[8,13,25] The two most pressing ICH investigational goals are: 1) early BP control and 2) hematoma volume reduction. Answering these questions would support decision-making, level-of-care choices, and the global research strategy of developing biologically informed treatments in general.[22]

Defining The Strategic Path: Consensus has developed slowly but firmly in favor of investigating the role of clot size reduction using less invasive methods and, perhaps, the use of disease-modifying biologics.[22,24,26] It is now recognized that "the mechanisms believed to play a part in brain injury induced by ICH differ in type, magnitude and timing from those of ischemic stroke."[22] In this environment, the MISTIE investigators

have produced some of the first encouraging data. The phase II findings utilized the path of biologically plausible animal models, validation via adaptive human studies, and advanced surgical consensus/data sharing, leading to an innovative image-based anatomically-targeted delivery of a biologic that modifies the clot and the interstitial space. The ICH SPRG recommendations prioritized a MIS trial. This goal, coupled with newly available MISTIE II trial evidence demonstrating proof-of-concept and surgical standardization in humans now offers substantial promise for mitigating a clinically beneficial portion of the primary injury in ICH. MISTIE III provides a major opportunity to gain critically needed knowledge about volume reduction and extravascular t-PA *and* produce an evidence-guided treatment for ICH.[8,22]

Remedying the Current State of Treatment: Prospective trial results will close the gap in treatment knowledge, define the benefit (or not) of MIS removal, and eventually assist with better decision-making. Until now, a Phase III trial, whose safety and feasibility are supported by animal data has not been undertaken.[27-29] The proposed trial, carefully developed over seven years of NIH funding to test the reliability of removal and the viability of the inclusion criteria, including the 72 hour time frame, can provide needed clinical and disease strategy answers. Outcome evidence can inform level-of-care decision-making for the choice of intervention and or the intensity of treatment. From the technical standpoint, results could demonstrate how to perform an explicitly defined, readily available, surgical technique that utilizes widely-available technology (CT, triage systems, & image guidance). This knowledge would be sufficient for broad national dissemination. From a biologic perspective, if the MIS+t-PA treatment translates current animal findings from clot size reduction to tissue preservation as a fully-tested human therapy, then this knowledge will build the foundation for a pipeline of biologically plausible injury-mitigating intervention(s).[22,30-33] And, rigorous data would greatly inform family decision-making based on individual preference in terms of both treatment options and long-term functional goals. Unique and reliable data from tools such as the Stroke Impact Scale[34-37] will help families as they make decisions based on personal health priorities (i.e., ability to live at home with independence) and individual patient wishes.[15,38]

Patient Population: Functionally independent (historical mRS of ≤ 1), male and female patients, who are age 18 to 80, with spontaneous, non-traumatic ICH with or without intraventricular hemorrhage (IVH), will be screened for enrollment. Radiographic imaging will be done to rule out underlying sources of bleeding other than hypertension. This specific group of patients will allow the investigators to determine if MIS+rt-PA can successfully reverse the brain-damaging effects of brain bleeding and return patients to functional baseline.

Method of Dosing: Subjects randomized to surgical management will receive 1.0 mg of rt-PA through the intraclot catheter every eight hours for up to nine doses. MISTIE II demonstrated that this dosage and route of administration is safe and balances clot dissolution against the complications of infection and symptomatic bleeding. Because the study is limited to nine or less doses, the total number of catheter openings will be similar

to or less than those in our previous IVH treatment safety study, which has an 8.3% rate of ventriculitis. MISTIE II further substantiated our choice of dosing every eight hours with a cerebral bacterial infection rate of 1%.

Choice of Control: The treatment group will be compared to the subjects receiving only conventional medical treatment. Subjects receiving MIS+rt-PA will be compared to an equal number of subjects randomized to receive conventional medical treatment. No vehicle controlled, placebo treated patients are planned in this study. This decision was made not to expose medically treated patients to the additional risk of surgical insertion of catheter. The medically treated patients do allow for the comparison of the intervention-induced complications that is the overall goal of this study, thus medically treated patients are the best overall control.

2.2 Supporting Data

Craniotomy for Superficial or Lobar Hematoma. STICH I was a negative trial where craniotomy was as safe as medical treatment and a small trend (2%-4%) mRS benefit favored surgery.[39] Because superficial (≤1 cm below the cortical surface) lobar hematoma locations possibly benefited from craniotomy,[20] STICH II tested the hypothesis that non-stabilized superficial lobar hematomas can undergo craniotomy safely and that surgery will produce a 12% benefit of improved functional outcome. Other post-hoc analyses of STICH I subgroups demonstrated that the deep location had a bad prognosis in both medically and surgically treated subjects; deep location subjects experienced the worst overall prognosis.[19,20] Additionally, there was no beneficial effect of early (<8 hr.; <24 hr.) craniotomy observed.[20] In fact, STICH demonstrated a trend that favored initiation of surgery after 24 hours compared to before 24 hours.[23]

MISTIE III Design and STICH II Results. Based on the published results of STICH II[101], the MISTIE III approach remains valid for lobar and deep hematomas. MISTIE III will continue to include the deep ICH location which opportunely is more common and has a stronger trend toward benefit. MISTIE III will test its innovative technique on hematomas at both locations where MISTIE II data has demonstrated benefit. STICH II, and MISTIE III when completed, will provide the missing human data to accept or reject clot removal in the overall strategy of tissue preservation and the care for the ICH subject.

Minimally Invasive Surgery (MIS) Volume Reduction and Outcome. A meta-analysis of world-wide clinical trials of craniotomy demonstrates a benefit for surgery over supportive care.[40] Importantly, a similar meta-analysis of MIS from China suggests an even stronger effect of the minimally invasive approach over a 72 hour time window.[24] A deficiency of scientific data exists from these studies on adequacy of or variation in surgical task performance.[30] The absence of data related to the surgical task, specifically extent of clot removed, limits the evaluation of a relation between outcome and volume removed.[41] MISTIE II *did* measure volume removed and correlated good outcomes with

greater removal of clot, making a removal strategy practical, safe, and reasonably promising.

Timing of Surgery (Other Trial Results and other Meta-Analysis Results). In the small number of trials with measures of volume reduction and functional outcomes, there is a remarkable consistency with the hypothesis that volume reduction is beneficial but not always technically optimal[41-46] and that a broad 72 hour time window exists.[24] Careful investigation suggests the stabilized subject in a non-emergent setting is the best candidate for removal. Neither ultra-early surgery (6hr)[47] nor the combination of factor VIIa and ultra-early surgery (<8hr) seem promising. In primary reports, the trends are for harm in the initial 6-8 hours.[23,47,48] Steiner's analysis of time to surgery shows a trend of benefit for the 24-72 hour time frame in contrast to outcomes when surgery was performed in the initial 24 hours as these early craniotomies were associated with additional hematoma growth and poorer outcomes.[48] Thus, safety analysis of the FAST data,[48] the experience of Morganstern and Grotta[47] point towards the 24-72 hour time frame. STICH showed a similar trend with harm if craniotomy was performed in the initial 8 hours, no benefit for craniotomy performed in the initial 24 hours, and a trend towards benefit for craniotomy if performed in the 24 to 72 hour time frame.[23] This is the time frame used in the proof-of-concept trials (3 MIS/1 Craniotomy) that have demonstrated benefit.[24,49,50,52] The MISTIE II positive outcome data utilized this timing which indicates that surgery performed on average after 6 hours of stabilizing hematoma growth, at a median time of 35 hrs, is associated with decreased tissue injury and improved functional outcome. The randomized medical subjects have the same ICU stay but a different acute and chronic course, experiencing 20 mL more edema by day 4, and 37 days greater care prior to return home, as well as fewer good mRS outcomes. The timing for surgery was tested in MISTIE II and represents the optimal window to both avoid hematoma growth and perform uncomplicated surgical removal.[48]

Medical Therapy Trial Results (Medical Therapy to Stabilize the ICH). Epidemiologic and trial-based data confirm that 15%-30% of subjects experience hematoma growth in the first 3-6 hours and represent a group at higher risk for poor functional outcome and mortality. This is consistent with the idea that bigger hematomas produce unwanted outcomes.[52] Unfortunately, neither ultra-early hemostasis (Factor VIIa)[53] nor ultra-early neuro-protection[54] led to improved outcome—in both cases the treated groups demonstrated small reductions in hematoma size compared to the untreated but these 1- to 4-mL differences were not associated with functional benefit. The FAST trial screening data suggest that 9% of all ICH subjects were in the ultra-early time frame and were candidates for "early stabilization." These data nicely show that the influence of hematoma expansion is limited to a small segment of the ICH population and also demonstrates that hematoma expansion of 2-8 mL (11%-26% of baseline volume) does not improve functional or mortality outcomes when tested in trials of 600 subjects.[53] These findings are in harmony with the hypothesis that cessation of bleeding (i.e. stabilization) provides no meaningful clinical benefit when pursued as the sole management goal. Meta-analysis of a similar, larger population from the VISTA database suggests that the threshold of ICH volume change required to produce functional

alteration is larger than the change produced in these "ICH growth" trials (a basement of about 6 to 12 mL of clot size change).[41,55] MISTIE III will produce a large volume change above this threshold.

Other Medical Trials (Testing for Benefit from Early BP Reduction). *ATACH II and INTERACT* are exploring the possible benefits of early stabilization and volume reduction via ultra-early control of blood pressure. Their post hoc analysis also finds a threshold of 6 mL of hematoma size change is needed to possibly alter mRS at 180 days.[41,56] MISTIE II produced early BP control (ED, 187/105; randomization, 145/72) in a manner similar to that of ATACH and INTERACT. If the ICH Guidelines are revised to include aggressive BP reduction based on the findings from INTERACT II, then its protocol will be incorporated into MISTIE III, to incorporate best practice. Stabilizing BP (and hematoma size) prior to enrollment will open treatment to a much larger set of ICH subjects, as more than 85% of subjects present after the ultra-early (<6 hr) time frame.[53,57] The MISTIE III protocol will skip the "ultra-early" time when MIS could interrupt the primary clotting process—plausibly based on the time needed for fibrin clot cross-linking providing fibrin chains stability and the formation of covalent links between fibrin and extracellular matrix proteins.[58]

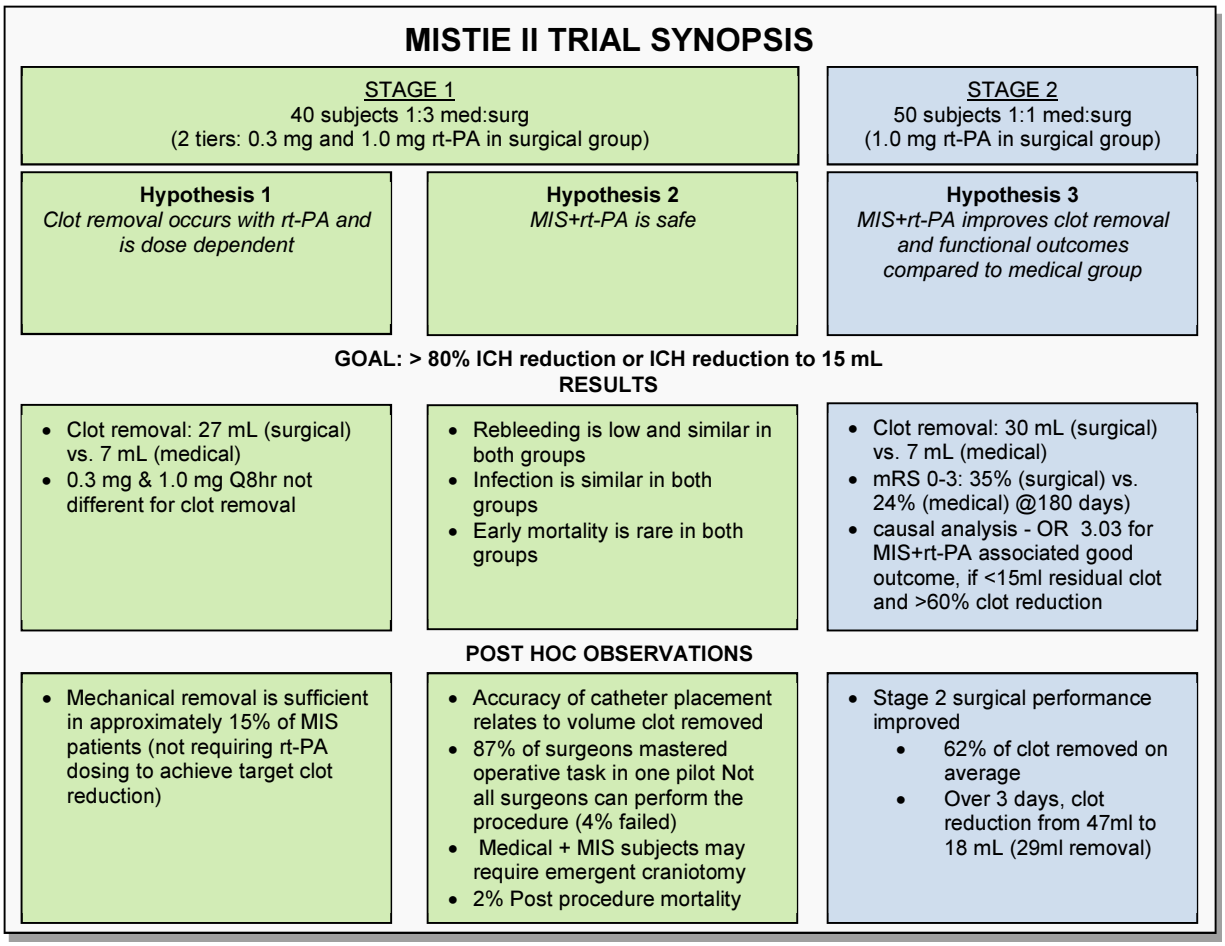


Figure 1. MISTIE II trial goals and post hoc observations. In Stage 1, 40 subjects were randomized 1:3 over two tiers. In Stage 2, 50 subjects were enrolled 1:1. Clot removal utilizing the MIS+rt-PA technique accomplished greater clot size reduction with improved outcome at 180 days as compared to the medical group. Recurrent bleeding, infection, and early mortality were low and similar in both groups.

Review of MISTIE II Data: The MISTIE II trial was a Phase II, two-stage trial of 96 randomized subjects with the overall goal of assessing the practical feasibility of image-guided, catheter placement and removal of clot from subjects with hypertensive ICH, as defined by absent vascular malformation and the presence of hypertension. Its goal was to provide proof that the same benefits that occurred in animals with rt-PA clot irrigation could be translated into humans. Results of MISTIE II hypotheses testing suggest successful translation of the technical aspects of removal and the putative benefit to humans. Specific information about the removal of clot from humans utilizing the MIS+rt-PA technique is summarized in Figure 1 as "Post Hoc Observations."

MISTIE II Trial Design*:* Stage 1 escalated the rt-PA dose (0.3 mg Q8hr, Tier 1 increased to 1.0 mg Q8hr, Tier 2) to test for dose response and safety. When compared to controls, both doses increased clot removal with no difference in bleeding rate (7% vs. 9%), leading to a decision to use the 1.0 mg dose. Tier 3 used the same precise image localization but a wider cannula and higher negative pressures (up to 300 mmHg) and is identified as the "ICES" Tier.[63] We report on 123 subjects (117 MISTIE & medical; 6 ICES, medical only).

MISTIE II Trial Safety*:* The MIS+rt-PA procedure was evaluated for safety by dose, trial stage and comparison to medically treated subjects as defined by the MISTIE protocol utilizing AHA ICH guidelines.[64] All emergent and ICU care was rendered according to guideline for each subject independent of randomization status. Of note, the influence of the withdrawal of care occurred equally among randomized subjects (surgical, 13%; medical, 10%).[15,65]

Mortality*:* Data on intention-to-treat (54 surgical, 42 medical, n= 96) and all subjects (27 pilot, 96 randomized, n= 123) are provided. Including pilots, 81 subjects underwent MIS+rt-PA. There were no intra-operative deaths. Seven-day mortality was chosen as the immediate post-operative period; mortality was 2% in the MISTIE II cohort, comparing favorably to the 7-day mortality in FAST (12%-14%). The two deaths were related to the severity of the primary bleeding event, with cause of death preoperative respiratory failure (case1) and pre-existing coronary artery disease leading to postoperative myocardial infarction (case2). Thirty-day mortality was 10% and 15% for the medical and surgical groups respectively. No differences were noted for mortality at any time point, for the intention-to-treat, or the total group (Fig. 2). Withdrawal of care was equal in each group (36% vs. 53%) as well as the withdrawal of care temporal profile.

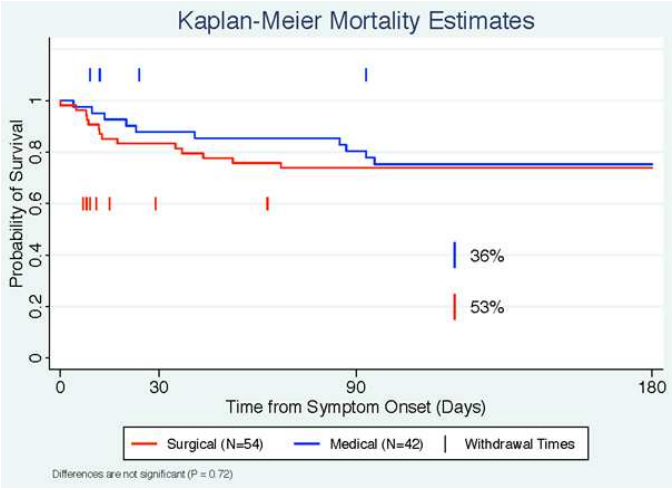


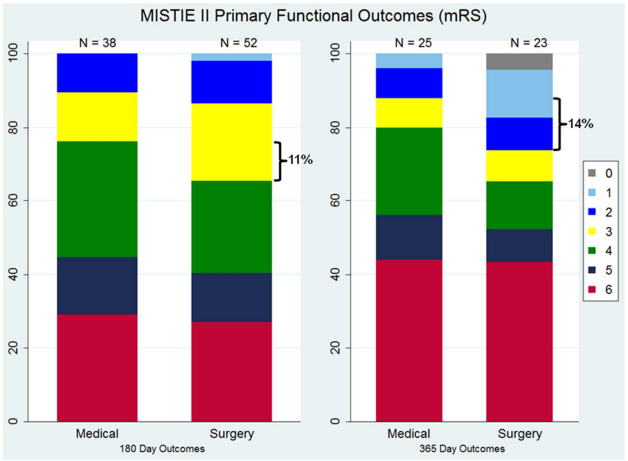
Figure 2. Kaplan Meier plot of mortality for randomized MISTIE II subjects. No differences were noted for mortality at any time point. Withdrawal of care was proportionally similar in both groups, and occurred at similar time frames in each group (Fischer's Exact Chi Square; non-significant).

Specified Safety Measures*:* Post-operative bleeding and infection occurred at low frequencies and below the literature-defined thresholds.[44,66] Two brain infections were observed: culture-negative ventriculitis, surgical subject and culture-negative meningitis, medical subject. Both resolved without consequence. Recurrent bleeding rate was 5% overall: surgical, 2.65% (CI 0.07, 13.5), medical, 6.2% (CI2.0, 13.8); pilot, 11%; randomized surgical, 3.7%; and randomized medical, 2.6%. It is difficult to attribute bleeding to the procedure or the drug; however, bleeding sites were frequently associated with the hematoma or catheter, preserving the need for caution of MIS+rt-PA as a trigger for increased likelihood of bleeding. MISTIE III will provide a better estimate of these rates. The overall rate of rebleeding (3.7%) compares well to the 10%-17% rate in other surgical and MIS trials.[49,50,66]

Edema: Edema is an early indicator of tissue injury[26,30,31,67] and is measured more easily in humans than cell death and ischemia.[68] In MISTIE II, the protocol prospectively tested

the idea that clot reduction would lead to edema reduction as is observed in animals[30,69,70] and preliminary data.[68] Analysis of perihematomal regions of MISTIE II surgical subjects, utilizing a validated method,[71] shows a reduction of 22 ml of edema when compared to medical subjects in the same time frame (% reduction of 22±35 % surgical vs. increase of 47±46% medical). This finding is consistent with the reduction of toxic metabolic injury seen in animal models[30,72] and inconsistent with a small number of prior "convenience samples" and clinical reports demonstrating an increase in edema after exposure to rt-PA.[73] The Phase III will confirm the consistency of benefit across a broad population, the degree to which hematoma and edema reduction relates to improved functional performance, and the possible cellular basis of a beneficial effect.

Functional Outcome Benefit: ICH recovery requires more time than recovery from ischemic stroke, with stable clinical and functional performance occurring at 180 to 365 days.[6,45,74] Surgical subjects achieved good functional outcomes more frequently than medical subjects, despite having a slightly worse initial ICH volume severity (34 mL vs. 43 mL), GCS score (12 vs. 11), and IVH size (2 mL vs. 4 mL). At 180 days, 35% of surgical subjects had reached mRS 0-3 compared to 24% in the medical group. When analyses were adjusted for initial severity imbalance, the effect increases. MISTIE II was amended to following the mRS 4, 5 subjects through 365 days. The differential benefit for the mRS 0-3 state increases to 14% and a significant proportion of mRS 0,1 and 2 states are observed in the surgical group where the difference between surgery and medical is also 14% (Fig. 3). Thus, an improvement across all levels of mRS appears to be associated with the MIS+rt-PA group and is consistent over time, with an important proportion reaching high degrees of independence. MISTIE III will confirm the reproducibility, size and generalizability of the benefit previously observed in the MISTIE II proof-of-concept trial. Subgroup analysis suggests no effect of location (deep vs. lobar), size, time to surgery or age (Fig. 4).



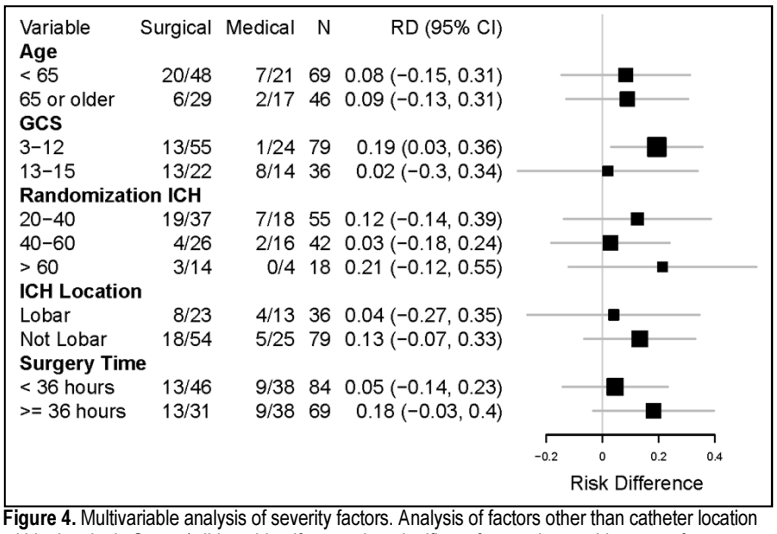


Figure 4. Multivariable analysis of severity factors. Analysis of factors other than catheter location within the clot in Stage 1 did not identify any other significant factors that could account for variation. This resulted in the simplification of instructions for the surgical task in Stage 2 regarding optimal catheter placement.

Surgical Performance and Functional Benefit: A range of clot size reductions occurred in the surgical group. Several reasons exist for this finding. Despite having set the goal of >80% clot reduction from the animal studies to the MISTIE II subjects, this goal was not well achieved at the MISTIE II sites in Stage 1. Initial evaluation of factors associated with clot size reduction suggests that the precision of the catheter location within the clot accounts for at least half of the variation (Fig. 5). Multivariate analysis of other factors, such as clot location, coagulation state, and age of the clot, did not identify a second critical factor; thus the instructions were simplified for the surgical task in Stage 2 and each site-surgeon was encouraged to replace catheters, if the initial placement was not optimal. Stage 2 results confirm the idea that a catheter more completely in contact with the clot will remove more blood (see Fig. 5 & Table 3). Prior to MISTIE II, no data existed describing the optimal amount of blood to remove or when to stop removing it. In 2004 the "a priori" goals of 80% clot size reduction and /or decrease clot size to $\leq$ 15ml to rectify the deficiency in surgical goals were selected. For this reason the MISTIE subjects are now evaluated with respect to the percent and absolute amount of blood clot removed (see Fig. 6). The odds ratio for a good result is enhanced (OR 3.04; CI 1.22, 8.03) if the MIS+rt-PA procedure removes more than 60% of the clot and produces end-of-treatment clot volume of 15 mL or less. Importantly, a causal analysis does not link the good outcomes in this "higher performance" surgical group to unequal (i.e., overly favorable) distribution of factors such as medical co-morbidities, age initial severity factors or clot properties.

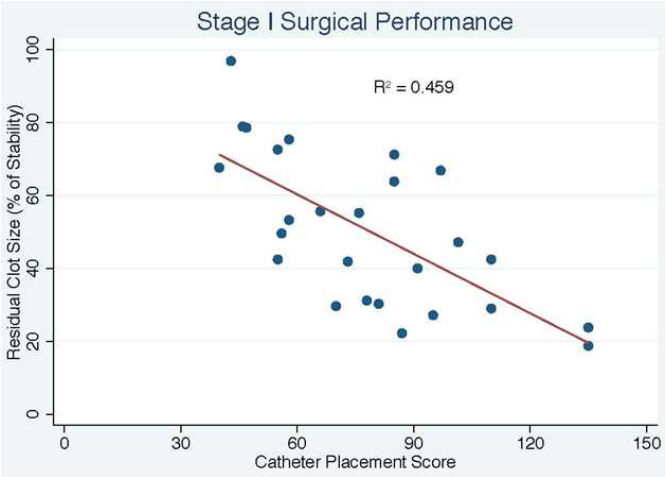


Figure 5. Correlation of Clot MIS+rt-PA catheter placement accuracy. Accuracy was the only factor related to extent of clot removal.

Table 1. Factors effecting functional outcome (n=90).

	Univariate Analysis	Multivariate Analysis	
	Odds Ratio (p-value)		
ICH Severity Parameters		**Model 1**	**Model 2**
Age	0.96 (0.029)	0.92 (0.004)	0.91 (0.002)
Stability ICH per 10 mL	0.65 (0.008)	0.85 (0.342)	0.95 (0.755)
Enrollment Total GCS Score	1.57 (< 0.001)	1.73 (< 0.001)	1.77 (< 0.001)
Surgical vs. Medically managed	1.71 (0.266)	2.73 (0.121)	NA
<15 mL remaining after treatment	2.65 (0.068)	NA	3.82 (0.056)
n=	90	90	90

Multivariate Model of Outcome: When well-established severity factors (ICH size, IVH size, presenting GCS, NIHSS) are considered, a multivariate model of outcome (mRS. 0-3 vs. 4-6), removal of clot is the third important factor in association with good outcome. Removal of clot has an OR of 0.27; (CI 0.066 to 1.07, p< 0.062), consistent with animal models as well as the primary hypothesis that removal of clot is beneficial. The factors that produce variability have now been adequately identified and this phase III trial is appropriately powered to definitively test for the benefits of MIS+t-PA and for the surgical importance of the clot volume reduction hypothesis.

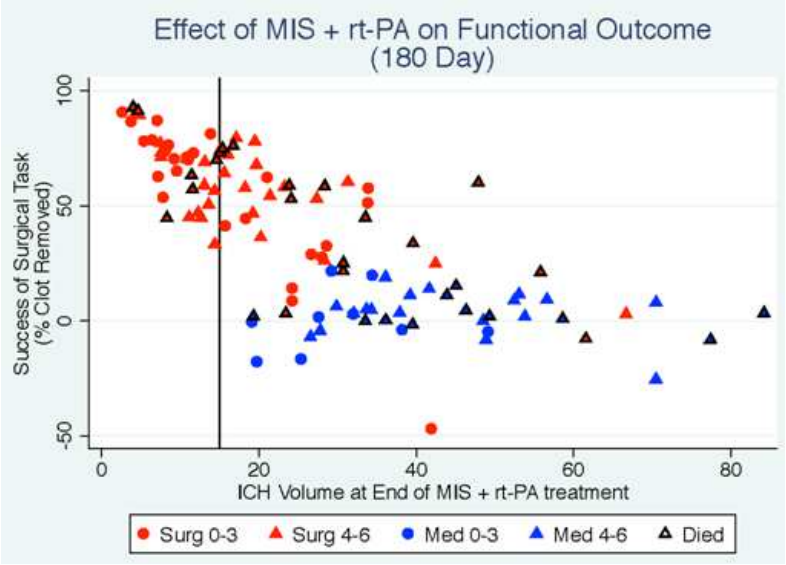


Figure 6. Relationship between percent clot size reduction with MIS+rt-PA vs. end of treatment clot volume demonstrates how change in clot size increases the likelihood of good outcomes. Medical subjects show minimal clot size reduction. A range of larger and smaller reductions is demonstrated for MIS+rt-PA subjects. Large percentage of clot reduction > 60% and end of treatment clot size < 15 mL (|) was associated with increased likelihood of mRS 0-3 (OR: 3.04).

Patient Utility: Besides mRS, the Barthel Index and Stroke Impact Scale (SIS) provide additional insights to post-stroke recovery. These include physical recovery, such as strength, hand function, ADL, mobility, emotion, communication, memory, thinking and social participation (all on a scale of 0 to 100 with a higher score indicating better recovery). MIS+rt-PA appears to improve physical ability compared to the standard treatment at 180 days. This is supported by SIS strength (mean difference = 11.8, SD = 7.6, p-value = 0.122) and mobility (mean difference = 10.3, SD = 8.8, p-value = 0.244) scales as well as by higher proportion of patients with independence in toilet use, sphincter control and mobility on Barthel scale in MIS+rt-PA group. Importantly, the observed mean differences are within the range of change regarded as clinically important.[75] Further, the memory score is also markedly higher in the MIS+rt-PA arm by 180 days post stroke

(mean difference = 8.8, SD = 8.3, p-value = 0.291). Although statistical significance was not achieved in these data, due to (suspected) small sample size in the standard treatment arm, the results suggest that MIS+rt-PA leads not just to better physical status, but also potentially to better quality of life (QOL) based on these measures. The MISTIE II data show that although the recovery of physical function and activities of daily living at 30 days is slow, other functions, such as emotion, memory and communication, respond to treatment earlier in the cohort. The average difference in SIS emotion and SIS-16 total score is 40.3, SD = 27.8, and the average emotion score at 30 days is 62.3, SD = 22.4. Social participation is most affected by altered physical function and has the lowest average score at 30 days. As expected, it increases in parallel to improvement in physical function at 180 days post stroke.

MISTIE III Innovation (Surgical Task and Trial Execution): Surgical centers with written feedback about each surgeon's task performance has produced uniform results in oncologic trials.[76-78] The outcomes of clinical trials testing surgical and skill-dependent therapies may be confounded by technical variations in the procedure and the skills and experience of the practitioner.[79] This could have happened in MISTE II, but did not. Both potential standardization problems were successfully addressed using innovative surgical center adjudication processes in MISTIE II. Until this NINDS trial, catheter location within the ICH had not been clearly demonstrated to play a critical role in the outcome of minimally invasive evacuations of ICH, nor had it been emphasized in previous publications on the safety and purported effectiveness of these techniques.[43] Not only did MISTIE II optimize the dose of thrombolytic, it defined, standardized and replicated the best surgical technique and catheter location strategies for optimal execution of the "MIS" surgical task. The process of standard surgical task description led rationally to the description of three specific trajectories with respective skull entry points for clots in three main brain locations. The sequential refinement of the surgical protocol resulted in enhanced clot evacuation and improved surgical outcome (See Table 1). Thirty-one surgeons used this technique without performance difference related to experience or frequency of performance. The surgery was standardized and applied in a coordinated manner following a brief, targeted training and achieved a uniform post-operative result. This is unique in its efficiency and innovative in its use of virtual teams. This program will again be utilized with well-defined MIS technical standardizations for the expanded group of sites needed for the trial. These tested and proven tools (87% proficiency following one pilot) will be deployed by the virtual Surgical Center to instruct new sites to maintain the same quality across the study period. If successful for 500 subjects and 90 sites, the investigators will have the road map for disseminating the protocol through leading clinical and research bodies, such as the AANS joint section on vascular neurosurgery and NINDS.[80]

What is the MISTIE Task & Can It Be Translated to Routine Practice? The MIS technique and its related image-guided catheter placement are universally practiced in treatment of tumors, functional disorders, aneurysms and hydrocephalus; techniques for each of these applications are performed in neurosurgical programs daily. The access to and prevalence of equipment for imaging and image guidance is equally universal. MIS

and catheter-based clot removal is a simple procedure and simpler than other MIS procedures using laparoscopic devices, robots, electrode sensors and/or remote-controlled manipulation of instruments. The investigators now have a 10-year clinical trial history of placing catheters for the delivery of rt-PA in a standardized manner. The obvious benefits of less operative trauma for the patient, less expense, shortened healing time, less pain and scarring, and less time in the operating room (OR) are universally attractive to patients, physicians and hospitals. Kojita has compared craniotomy (175 min) to MIS (45 min) and confirmed the simplicity of this procedure. In MISTIE II, the surgery total time was 56 minutes (± 31 min) with a median time of 48 min; the 1st and 2nd stage medians were 51 and 44 minutes respectively. To be broadly adopted, "directive evidence" needs to exist, thus the safety and effectiveness of the procedure must be demonstrated with a randomized controlled trials (RCT). The simplicity of MIS creates the opportunity for such a trial and subsequent broad adoption.

Surgical Center: The trial's Surgical Center is the critically important trial management innovation our team perfected in the MISTIE II and CLEAR III trials. Innovations critical to standardization, not previously utilized in surgical trials such as STICH, include: 1. training modules for credentialing surgical investigators; 2. quarterly "Surgical Matters that Matter" updates based on emerging experience from ongoing cases; 3. collegial surgical review, telementoring, and telemonitoring of cases at screening and enrollment, as needed, including operative planning with dialogues on burr hole location and catheter placement trajectory; 4. uniform external review of surgical performance for each enrolled subject and feed-back about protocol deviations; and 5. ongoing reviews of adverse events vs. surgical morbidity, and protocol optimization. Written reporting and review have led to uniform surgical results in oncologic surgical trials.[76-78] The novel structure of real-time, web-based quality assurance has not, to the knowledge of the investigators, been used in any neurologic/neurosurgical trial prior to CLEAR III and MISTIE II; it has been very effective in establishing the innovative and essential catheter trajectory calibration and prospectively testing the importance of end-

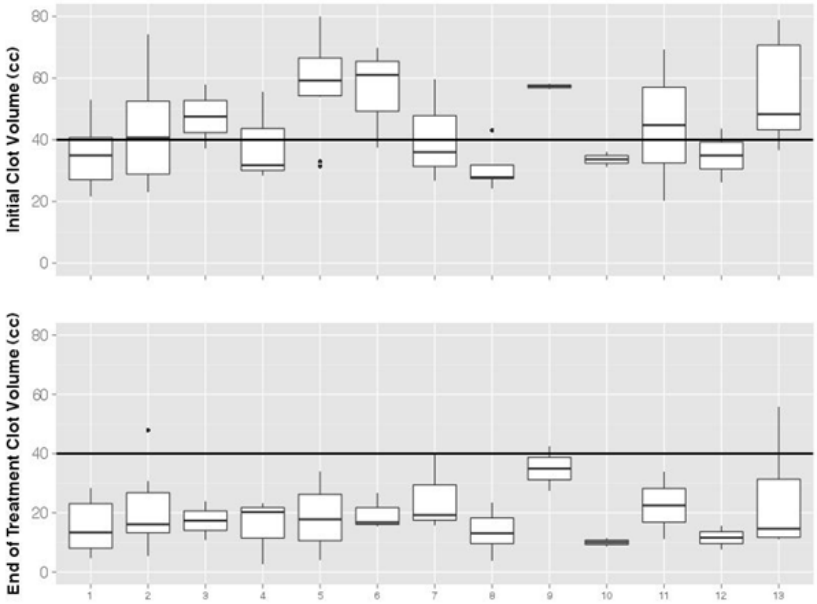


Figure 7. Individual surgeon performance (n=57 subjects). These box plots define the median 25th, 75th percentiles of pre-surgical and end-of-treatment clot volume. All surgeons were able to accomplish reduction from > 40 mL to < 20 mL.

of-treatment volumes. The use of a virtual surgical center has been highly productive and successful.

Volume Reduction & Catheter Trajectory Efficacy*:* A wide range of clot size reductions occurred in the Stage 1 surgical group despite the goal of >80% clot reduction. Initial evaluation suggested that precision of catheter location within the clot accounts for at least half of the variation. Utilizing univariate and multivariable regression no other significant critical factors such as clot location, coagulation state, and age of the clot could be identified; thus, the instructions for targeting the clot were simplified and surgeons were encouraged to replace catheters if the initial anatomic placement was not optimal. Stage 2 results confirm that catheters more completely in contact with clots will remove more blood (see Fig. 5). In addition to accurately engaging the clot, trajectory analysis by the principal surgical investigator and the central image center determined that all Stage 1 catheters could have been advanced by one of three approaches. The DSMB approved the trajectory design change and three specific trajectories were added to the surgical task description and used in Stage 2 with improved results. Data demonstrates improved removal; with an average of 29 $\pm$15 mL absolute volume of blood removed representing 63% reduction in clot size (Table 3). The proportion of subjects experiencing reduction to 15 mL or less also increased from 39 to 48%. This explicit task description, which is now validated against the surgical goal, has led to a standardized surgical intervention. All elements of surgical technical performance improved in Stage 2, including: operative duration, operative efficiency of catheter trajectory, clot targeting, percentage of subjects achieving >60% removal, and percentage of patients achieving clot reduction to 15 mL or less.

Table 3: Surgical Performance Results for Stage I and II MISTIE Subjects. Simplifying the definition of the surgical task after Stage I improved the percent of subjects achieving 60% removal and end of treatment volume < 15 mL.

		Baseline Vol	Vol Removed	Removed %	EOT Vol	pts EOT < 15 (%)	pts with > 60% Removal (%)
Medical	Stage 1	43 ± 16	4 ± 3	**9**	39 ± 15	0	0
n= 42	Stage 2	43 ± 15	2 ± 9	**3**	41 ± 17	1(3%)	1(3%)
Surgical	Stage 1	49 ± 23	26 ± 19	**51**	22 ± 12	11(39%)	10(36%)
n= 53	Stage 2	47± 16	29 ± 15	**63**	18 ± 14	12(48%)	18(72%)

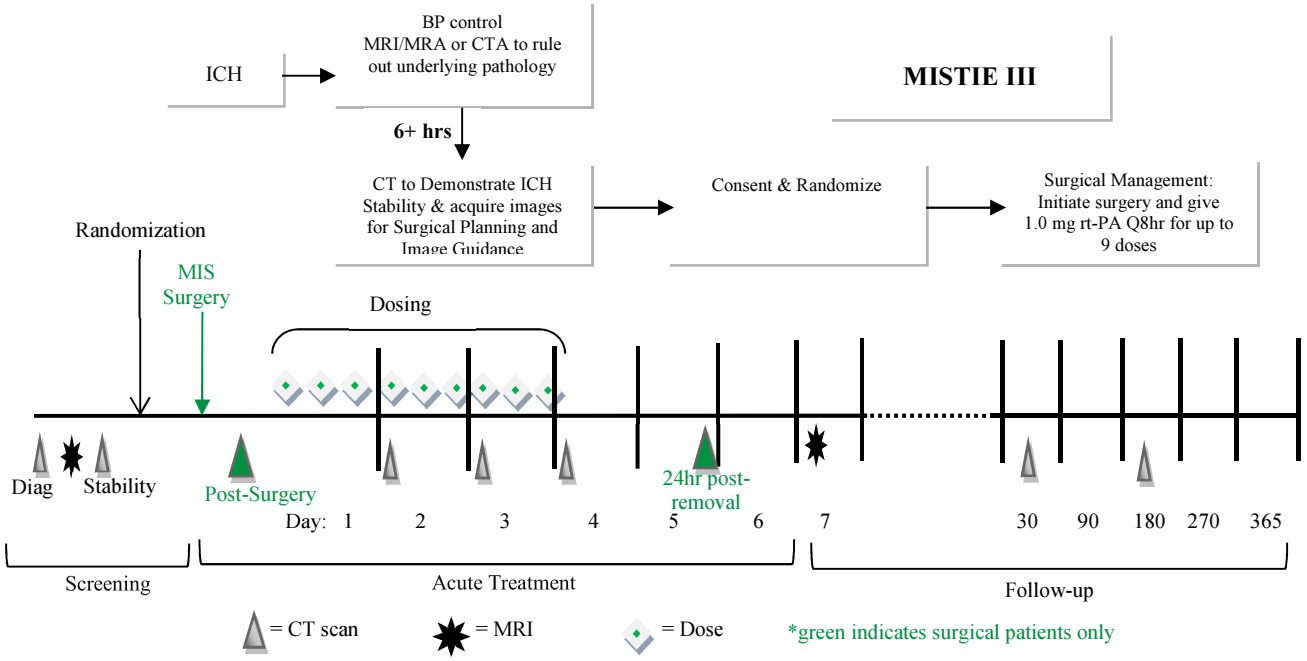
*All volumes mean ± SD(mL), Pts=Patients, Vol=Volume, EOT=End of Treatment

Reproducibility of Surgical Results: Improved catheter localization after completion of Stage 1 was a function of explicit trajectory planning. Specifically, sequence and number of procedures conferred no clot removal performance advantage. Analysis of sequential performance showed neither ongoing improvement nor a threshold of multiple subjects operated on before adequate clot removal. The MISTIE II data support the assumption that, via this proof-of-concept trial the investigators have: 1) adequately defined the surgical task; 2) identified routine variations in standard surgical practice (across sites and operations) that might limit the effectiveness of surgery; 3) trained the trial surgeons to avoid these pitfalls; 4) a single pilot is an effective test of surgical capability and 5)

identified stopping rules for the surgical goal associated with the primary hypothesis of safe clot size reduction. No adverse events associated with these surgical goals have been observed. MISTIE III will provide robust data to test supporting or rejecting these surgical procedures, their associated goals, and the adequacy of simple surgical training.

3 STUDY DESIGN

This study is a phase III, randomized, open-label, multicenter evaluation of MIS and ICH lysis with rt-PA versus medical care. The study (n=500) will evaluate the efficacy and safety of MIS plus 1 mg of rt-PA administered every eight hours for up to nine doses as compared to subjects treated with conventional medical management. Outcome assessment will be performed by local certified investigators and adjudicated by central blinded investigators.



Subjects enrolling in this study may also consent to participate in an ancillary study titled *Mechanisms of Tissue Injury in MISTIE III*. This ancillary study offers a tremendous opportunity to leverage clinical trial data to bring novel insights from ICH pathophysiology into the clinical realm, using neuroimaging, genetic and inflammatory markers of disease to provide clinicians with powerful new tools to guide surgical therapy and develop new therapeutic targets. See Appendix 8 for the ancillary study protocol.

4 SELECTION AND ENROLLMENT OF SUBJECTS

4.1 Inclusion Criteria

4.1.1 Spontaneous supratentorial ICH ≥ 30 mL measured by the site utilizing ABC/2 method using radiographic imaging (CT, CTA, etc.), with a GCS ≤ 14 or a NIHSS ≥ 6.

4.1.2 Stability CT scan done at least 6 hours after diagnostic CT showing clot stability (growth < 5 mL as measured by ABC/2 method).

If the clot volume measured on this stability CT scan increases by 5 mL or more, a second stability determination is allowed by repeat CT scan at least 12 hours later. Additional scans are permitted as needed every 12 hours to continue to monitor for stability up until the eligibility time window closes. Subsequent clot retraction remains inclusionary as long as the ICH clot size remains ≥ 25 mL.

4.1.3 Symptoms less than 24 hours prior to diagnostic CT (dCT) scan. An unknown time of onset is exclusionary. Use the time the patient was last known to be well for patients that awaken from sleep with symptoms.

4.1.4 Ability to randomize between 12 and 72 hours after dCT.

4.1.5 SBP < 180 mmHg sustained for six hours recorded closest to the time of randomization.

4.1.6 Historical Rankin score of 0 or 1.

4.1.7 Age ≥ 18 and older.

4.2 Exclusion Criteria

4.2.1 Infratentorial hemorrhage.

4.2.2 Ruptured aneurysm, arteriovenous malformation (AVM), vascular anomaly, Moyamoya disease, hemorrhagic conversion of an ischemic infarct, recurrence of a recent (< 1 year) hemorrhage, diagnosed with radiographic imaging.

4.2.3 Patients with unstable mass or evolving intracranial compartment syndrome.

4.2.4 Irreversible impaired brain stem function (bilateral fixed, dilated pupils and extensor motor posturing), GCS ≤ 4.

4.2.5 Thalamic bleeds with apparent midbrain extension with third nerve palsy or dilated and non-reactive pupils. Other (supranuclear) gaze abnormalities are not exclusions. Note: Patients with a posterior fossa ICH or cerebellar hematomas are ineligible.

4.2.6 Intraventricular hemorrhage requiring treatment for IVH-related (casting) mass effect or shift due to trapped ventricle. EVD to treat ICP is allowed.

4.2.7 Platelet count < 100,000; INR > 1.4.

4.2.8 Any irreversible coagulopathy or known clotting disorder.

4.2.9 Inability to sustain INR ≤ 1.4 using short- and long-acting procoagulants (such as but not limited to NovoSeven, FFP, and/or vitamin K).

4.2.10 Subjects requiring long-term anti-coagulation are excluded. Reversal of anti-coagulation is permitted for medically stable patients who can realistically tolerate the short term risk of reversal. Patient must not require Coumadin (anticoagulation) during the first 30 days, and normalized coagulation parameters must be demonstrated, monitored closely and maintained during the period of brain instrumentation.

4.2.11 Use of Dabigatran, Apixaban, and/or Rivaroxaban (or a similar medication from the similar medication class) prior to symptom onset.

4.2.12 Internal bleeding involving retroperitoneal, gastrointestinal, or genitourinary site or respiratory tract bleeding.

4.2.13 Superficial or surface bleeding, observed mainly at vascular puncture and access sites (e.g., venous cutdowns, arterial punctures, etc.) or site of recent surgical intervention.

4.2.14 Positive urine or serum pregnancy test in pre-menopausal female subjects without a documented history of surgical sterilization.

4.2.15 Allergy/sensitivity to rt-PA.

4.2.16 Prior enrollment in the study.

4.2.17 Participation in a concurrent interventional medical investigation or clinical trial. Patients in observational, natural history, and/or epidemiological studies not involving an intervention are eligible.

4.2.18 Not expected to survive to the day 365 visit due to co-morbidities, or are DNR/DNI status prior to randomization.

4.2.19 Any concurrent serious illness that would interfere with the outcome assessments including hepatic, renal, gastroenterologic, respiratory, cardiovascular, endocrinologic, immunologic, and hematologic disease.

4.2.20 Patients with a mechanical heart valve. Presence of bio-prosthetic valve(s) is permitted.

4.2.21 Known risk for embolization, including history of left heart thrombus, mitral stenosis with atrial fibrillation, acute pericarditis, or subacute bacterial endocarditis. Atrial fibrillation without mitral stenosis is permitted.

4.2.22 Any other condition that the investigator believes would pose a significant hazard to the subject if the investigational therapy were initiated.

4.2.23 Active drug or alcohol use or dependence that, in the opinion of the site investigator, would interfere with adherence to study requirements.

4.2.24 In the investigator's opinion, the patient is unstable and would benefit from a specific intervention rather than supportive care plus or minus MIS+rt-PA removal of the ICH.

4.2.25 Inability or unwillingness of subject or legal guardian/representative to give written informed consent.

4.3 Study Enrollment Procedures

4.3.1 **Screening Procedures**

1. Diagnostic CT (dCT) scan. This scan is defined as the first CT scan performed that is used to diagnose the ICH. At each study center ICH volume will be determined in the following manner: On the CT slice with the largest area of ICH, the largest diameter (A) of the hematoma will be measured in centimeters. The dimension of the hemorrhage perpendicular to the largest diameter will represent the second diameter (B) in centimeters. The height of the hematoma will be calculated by multiplying the number of slices involved by the slice thickness, providing the third diameter (C). The three diameters will be multiplied and then divided by two (AxBxC/2) to obtain the volume of ICH in cubic centimeters.

2. Stability CT scan. This scan will be done at least six hours after the dCT scan to determine clot stability. The clot volume measured using the technique described above must not increase from the volume measured on the dCT scan by 5 mL or more. Fiduciary markers should be placed at the time of this scan if the patient appears to be eligible (or suitable anatomic landmarks noted). Fiducials should remain in place until after the post catheter insertion CT scan for those subjects randomized to surgical management.

If the clot volume measured on the first stability CT scan (at least 6 hours after initial/diagnostic scan) increases by 5 mL or more, a second stability determination is allowed by repeat CT scan at least 12 hours after the previous stability scan. Additional CT scans are permitted as needed at least every 12 hours to continue to monitor for stability up until the eligibility time window closes.

3. Imaging to rule out underlying pathology. A CTA will be done prior to randomization, preferably at the time of the stability CT scan, to rule out underlying cerebro-vascular or brain pathology. If a CTA is contraindicated due to renal impairment, an MRA will be done at this time instead. In addition, an MRI will be done at baseline and repeated on day 7-10 (± 1 day) to assess edema and cerebral ischemia and contain the following sequences: T1, MPRAGE, DWI, AXIAL FLAIR, AXIAL SWI, PWI, Axial T1 POST, and DTI. In cases where SWI of sufficient quality cannot be adequately obtained, T2W GRE may be substituted. B0 and ADC Maps should be uploaded along with the DWI B-100 images. Obtaining the baseline MRI prior to first dose of rt-PA is preferred, or obtain any time on days 1-3 per scanner availability. The requirement to obtain either MRI is waived for study centers located in Spain.

CT angiogram or routine angiogram with evaluation for "spot sign" is encouraged and considered standard of care to complete the evaluation for aneurysm, AVM, or other malformations if the CTA or MRA are inconclusive.

4. Blood pressure. Blood pressure stability is defined as SBP < 180 mmHg sustained over six hours prior to randomization.

5. NIHSS. A NIHSS score must be obtained and must be ≥ 6 (or a GCS of ≤ 14) for the patient to be eligible (using distal motor function). The NIHSS must be done by a certified examiner. The NIHSS must be done at the time of enrollment to confirm eligibility.

4.3.2 **Tracking Procedure.** All study center investigators and study coordinators must have an established relationship with their emergency department personnel and must be routinely notified of hemorrhagic and ischemic strokes. Each center will design a system for patient tracking that best suits its needs according to time, personnel, and the patient population. The study coordinator will be responsible for tracking subjects and scheduling appointments. The study coordinator will inform subjects of the follow-up expectations when informed consent is obtained, and will maintain contact through telephone calls and letters. The Clinical Coordinating Center (CCC) database will drive a monthly report and centers will receive emails listing subjects due for assessment and overdue for assessments. The study coordinator will be required to document in the VISION EDC system whenever subjects are lost to follow-up or assessments are overdue. A subject is only considered "lost to follow-up" if contact is not achieved at the day 365 visit.

Attempts to find and establish contact with a subject must be made at every follow-up time-point, even if unsuccessful at an earlier time-point. A subject lost to follow-up will not be tolerated; in such case the site investigator will be placed on a remediation plan to improve subject tracking.

4.3.3 **Facilities.** To be eligible as a site, a center must demonstrate uniform referral, triage, and medical management practices. Each center must have emergency stroke transport services, stroke triage screening, a full time neurovascular neurosurgeon, and a full-time stroke research coordinator dedicated to this trial. To assure standardization of technical capabilities, the study chairman and appropriate CCC administrators will review each site's triage capabilities, emergency department facilities, pharmacies, imaging resources, and neurological ICUs. The Executive Committee (EC) along with approval from NINDS is ultimately responsible for the selection of the sites and investigators. In addition to these site criteria evaluated by the CCC, each site must designate a Surgical co-PI or Lead Surgeon, with an additional surgeon designated as a back-up, who will oversee all MISTIE cases, act as a liaison with the trial leadership on surgical matters, and who will help coordinator the credentialing of site surgeons who will perform the MIS procedure. MISTIE qualified surgeons at each site must be identified and individually credentialed by the trial's Surgical Center. This includes the demonstration of previous experience with the MIS procedure, current active surgical privileges in stereotactic neurosurgery, and the successful completion of a mandatory Surgical Center initiation conference on surgical protocol and procedure.

4.3.4 **Documentation for ineligibility.** Monthly reports of subject accrual (enrolled and screened but not enrolled) and other protocol compliance data will be provided by the CCC. All patients with ICH, whether eligible or not, who have been screened by study personnel at participating hospitals will be documented in the VISION EDC system. All reasons for exclusion for each patient not entered into the trial will be recorded. Each participating hospital will enter screening data into the VISION EDC system daily for review of screening and eligibility performance. Once all fields are completed, or an inclusion/exclusion criterion is failed, the system will either document the subject as a screen failure or prompt the coordinator to randomize the eligible subject.

Study centers failing to enroll over 5-7 months will undergo remediation with possible termination. Study centers in this situation may appeal to the CCC. If the study center can present a strong case for extenuating circumstances, then the site will remain in the trial for up to 9 months. At 9 months, study centers will be placed on probation with a final opportunity to enroll or be closed at 12 months.

4.3.5 **Informed consent.** The informed consent document will be used to explain the risks and benefits in simple terms to the patient or authorized representative

before the patient is entered into the study. The informed consent document must contain a statement that the consent is freely given, that the patient/authorized representative is aware of the risks and benefits of entering the study and the patient is free to withdraw from the study at any time. A sample informed consent form for all sites is included in Appendix 1 with an additional HIPAA template for international enrolling centers in Appendix 2.

The Investigator or designee is responsible for obtaining informed consent from each patient or their authorized representative and for obtaining the appropriate signatures and dates on the informed consent document prior to the performance of any protocol procedures and prior to the administration of study drug. Informed consent by an authorized representative of the patient should be obtained according to the clinical judgment of the investigator.

4.3.6 **Randomization.** Patients who meet all of the inclusion and exclusion criteria using the above screening procedures and who provide consent will be randomized to conventional medical management or surgery (MIS+rt-PA).

For those subjects randomized to surgery (MIS+rt-PA), the operative procedure should occur as close as possible to the time of randomization. If the surgical procedure is postponed to accommodate scheduling (i.e., it is preferable to wait until 6 am instead of midnight), obtain a CT scan to re-confirm stability of the ICH and re-confirm blood pressure stability prior to beginning the surgical procedure. If either or both are unstable, refer to page 23, Stability CT scan for clot stability and page 27, Cardiovascular management for BP stability.

The first dose of study drug is administered six or more hours after the surgical procedure and only after surgical center review.

5 STUDY INTERVENTIONS

5.1 Interventions, Administration, and Duration

All subjects will be followed daily for six days post randomization. All subjects will have an MRI (unless contraindicated) performed once at day 7-10 (± 1 day) or hospital discharge, whichever occurs first, to compare with the baseline MRI (if done) to measure edema. The requirement to obtain MRI is waived for study centers located in Spain. See section 4.3.1 Screening procedures, item 3 above for specific sequences.

All subjects will be required to return for a follow-up clinic visit at days 30 (± 7 days), 180 (± 14 days), and 365 (± 14 days). A telephone follow-up will be done at days 90 (± 7 days) and 270 (± 14 days).

5.1.1 Medical Management: All Subjects. Subjects in both groups, medical management and surgical management, will be treated medically using standard

ICU protocols (Appendix 3). This includes but is not limited to the following guidelines:

1. Intracranial pressure (ICP) management. Placement of an ICP monitor is recommended for subjects demonstrating obtundation, which we define as GCS ≤ 8 on a minimum of two observations over eight hours. ICP monitoring device selection is the discretion of the treating surgeon; however, the Camino parenchymal catheter has been pre-specified as the device of choice for the trial. The non-emergent ICP monitor would ideally be placed prior to rt-PA administrations or at least six hours after dosing. A new CT scan must be obtained after ICP monitor placement to assess stability of the current hemorrhage and to monitor for any new bleeding. If ICP is monitored, nursing assessments and ICP monitoring will be performed on a Q4hr basis, as will routine zeroing and recalibration of the system. The goals of ICP management are to sustain intracranial pressure below 20 mmHg and to improve the patient's level of consciousness.

2. Neurological status will be assessed Q4hr using GCS scoring. A neurological deterioration (neuroworsening) will be defined as any GCS decrease of greater than two points on the motor scale sustained for eight hours without sedation and is required to be reported as an AE/SAE. Daily attempts to discontinue sedation will be made. A daily neurologic exam is recommended to be coordinated with this attempted sedation withdrawal.

3. Cardiovascular management. The patient's blood pressure must be stable to be eligible for randomization. Blood pressure stability is defined as SBP < 180 mmHg for a period of six hours. This six-hour period must be maintained and documented as close to but prior to randomization as possible. Blood pressure management should conform to current AHA guidelines to maintain SBP < 180 mmHg throughout the first 6 days of the ICU stay to reduce the risk of bleeding events. The systolic and diastolic pressures over the six-hour monitoring period should be documented in the medical record as source documentation.

Current AHA Guidelines (Morgenstern 2010):

1. If SBP is >200 mm Hg or MAP is >150 mmHg, then consider aggressive reduction of BP with continuous intravenous infusion, with frequent BP monitoring every 5 min.
2. If SBP is >180 mm Hg or MAP is >130 mmHg and there is the possibility of elevated ICP, then consider monitoring ICP and reducing BP using intermittent or continuous intravenous medications while maintaining a cerebral perfusion pressure ≥60 mmHg.
3. If SBP is >180 mmHg or MAP is >130 mmHg and there is no evidence of elevated ICP, then consider a modest reduction of BP (eg, MAP of 110 mmHg or target BP of 160/90 mmHg) using intermittent or continuous intravenous

medications to control BP and clinically reexamine the patient every 15 min.

4. Respiratory care will be directed at promoting adequate oxygenation without airway compromise, with full pulmonary inflation, and with oxygenation ≥ 90% on room air or supplemental O_2 by face mask of 28% or less.

5. Nutritional support will consist of optimal calories, defined as ≥ 30 kcal/kg and 1.5 gm protein/kg. Feeding will be achieved by the least invasive means necessary, but with the goal of reaching full nutritional support by no later than day 7 of illness.

6. Deep venous thrombophlebitis and pulmonary embolus prophylaxis will be undertaken on the day of admission with the use of sequential compression devices (SCDs). For patients at high risk of thromboembolism, study center standard of care policies may govern the use of low molecular weight, fractionated and unfractionated heparins for DVT prophylaxis during the acute treatment and follow-up periods (criteria established by the American Orthopedic Association).

7. Withdrawal of care discussions of prognosis and decisions to continue or limit, or to withdraw, life-sustaining interventions will be conducted according to each institution's policies for end-of-life decision-making, as well as their institutional codes of medical ethics. The study assumes any such discussion will reflect the patient's wishes and the known facts regarding prognosis. Where the PI is not the managing physician it is assumed that those individuals will confer prior to presentation of the consensus prognosis and planned course of treatment. In some situations, the investigator may choose to select a colleague to serve in the clinician role or request a review by the hospital's ethics committee or other knowledgeable expert.

5.1.2 Experimental Intervention: MISTIE-Surgery. A neurosurgeon credentialed by the Surgical Center will perform the procedure. Credentialing shall include successful participation in MISTIE II or the review of at least one case of a MISTIE-type intervention by the surgeon outside of the trial, verification of hospital privileges in stereotactic and image-guided procedures, and documentation of viewing a **surgical standardization** presentation of the MIS procedure to insert the catheter is mandatory before credentialing a center's neurosurgery personnel. A PowerPoint presentation has been produced describing the catheter placement procedure and apparatus, sterile field techniques and the exact process for aspirating the clot. The presentation is available on the trial website (www.braininjuryoutcomes.com). It will be used continuously to train and retrain personnel performing the surgery to assure the standardization of surgical procedure. This presentation will be edited as new safety data are developed. Each site will maintain a log of eligible surgeon(s) along with the date and time of viewing. Each credentialed surgeon must also complete a mandatory

Surgical Center teleconference to review the surgical protocol and technical aspects of the procedure.

Optimal trajectory determination: The neurosurgeon will review a 3D reconstruction of the ICH on the CT scan to determine the burr hole location, catheter trajectory, and hematoma target to be used during the operative procedure. The neurosurgeon will select the representative slices reviewed for trajectory determination and the coordinator will submit the full set of DICOM (digital imaging and communications in medicine) images for review. The images will be uploaded to the EDC system, the surgical review form will be completed by the neurosurgeon or coordinator in the EDC system and both will be reviewed by the Surgical Center. Burr hole location, trajectory determination, and target will be coded as A, B, or C.

Option A is used for a deep-seated ICH occupying the anterior third of the basal ganglia with a typical "oval" shape (American football shape). A type A ICH should have an entry point in the low anterior frontal area frequently close to the midline near the eyebrow, and the trajectory of the catheter must be along the longitudinal axis of the clot.

Option B is used for a deep-seated ICH occupying the posterior third of the basal ganglia with a more roundish to elliptical shape. A type B ICH should have an entry point in the posterior parietal-occipital area, almost always several centimeters lateral from the midline to avoid the occipital ventricular horn, and the trajectory of the catheter has to be along the longitudinal axis of the clot.

Option C is used for superficial (lobar) ICH with variable shape, but is often more spherical. A type C ICH should have an entry point at the superficial area closest to the clot. This is a skull entry point sitting on the widest "equatorial point" of a spherical-shaped clot. The trajectory of the catheter has to be along the widest, or "equatorial", axis of the clot.

Surgical Center review of optimal surgical plan: The Surgical Center personnel will perform real-time (within six hours of data submission) review of 3D images to instruct the site that the proposed burr hole location and trajectory are appropriate or that a different location/trajectory is recommended. Feedback of the results of their review is documented in the EDC. The site neurosurgeon will proceed with the proposed surgical plan or the Surgical Center recommended plan. If there is disagreement between the two surgical plans, the site neurosurgeon has to demonstrate the rationale of his/her plan before using a surgical plan different from that proposed by the Surgical Center. See the Manual of Operations and Procedures (MOP) for a detailed description of personnel involved, responsibilities, and contact information.

Catheter placement: Antibiotic therapy should be administered pre-operatively (hospital protocol or 1-2G Ancef IV; dose is subject-weight dependent) then repeated every eight hours until the catheter is removed (hospital protocol or Ancef IV 1 G Q8hrs). If the subject has a known or suspected penicillin drug allergy, then antibiotic coverage will be administered pre-operatively and continued with each institution's non-penicillin drug of choice until the catheter is removed.

The procedure will be performed in the operating room, procedural CT or MRI scanner, or the ICU. After administration of the appropriate anesthetic, a Mayfield headrest is secured to the subject's head. A reference device is clamped to the Mayfield headrest. The image guidance system unit must be in direct line to the table with no line-of-sight obstruction. Registration is completed by correlating six points on the subject's head to six points on the previously loaded CT scan. Verification of accuracy is accomplished by testing various known landmarks on the subject's face to the image on the computer monitor. Re-registration during the case is accomplished as needed by repeating the correlation of the six landmarks on the subject's head to the CT scan. The procedure is completed in the usual sterile manner for burr hole and catheter placement. Other forms of image guidance which are acceptable include stereotactic robotic arms, electromagnetic tracking without skull fixation (only under general anesthesia and pharmacologic paralysis), or direct "real-time" image guidance in procedural CT or MRI.

The site of the entry burr hole is determined using radio-opaque dot localization if a standard frontal burr hole is insufficient. Standard frontal burr holes will be placed 3 cm lateral to the midline, anterior to the coronal suture for ipsilateral frontal, capsular and thalamic hematomas. If the subject has a deep brain hemorrhage (Options A and B), a large frontal burr hole will be used. If a lobar hemorrhage (Option C), the burr hole will be placed over the affected lobe. The position of the burr hole should be made posterior to the thickest portion of the hematoma. Surgical considerations regarding eloquent tissue and hematoma shape and location may require other burr-hole locations to optimize trochar/catheter trajectory to the target. A one-inch incision will be made in the scalp. The burr hole is drilled and the dura is opened with a small incision.

After the proper process of registration and localization with the image guidance system an introducer cannula will be placed stereotactically into the center of the hematoma. Up to two rigid cannula passes will be allowed to minimize morbidity from the catheter implantation. The introducer portion is then removed and careful hematoma aspiration is performed free hand using a 10 cc syringe until there is no longer any fluid component of the clot noted in the aspirate and/or until first resistance. Multiple aspirations may be used to meet these criteria. Volume aspirated will be documented. Following completion of hematoma aspiration, a soft ventriculostomy catheter is then passed through the

rigid cannula and then the rigid cannula is removed leaving the soft catheter with all its perforations in the center of the residual hematoma. Tunnel the catheter subcutaneously away from the incision. The catheter is then connected to a three-way stopcock and then to a closed drainage system.

A CT scan should be done at this time to confirm correct placement, using windowing to view the side ports of the catheter, and measure clot size reduction as compared to the volume measured on the stability CT scan. The catheter should be placed 2/3 of the way along the longest axis of the clot and in the middle of the width of the clot (i.e., within the middle 2/3 of the diameter). The **Surgical Center** will review this CT scan to confirm adequate catheter position prior to rt-PA administration. This review is repeated after any catheter adjustment or placement.

After placement of the catheter and a CT scan to confirm correct placement, a six-hour post-surgical stabilization period is required prior to first injection of rt-PA. Keep the drainage system to drainage for six hours post catheter placement ***prior*** to first dose of rt-PA. This time is mandated to reduce the possibility of secondary hemorrhage. If new bleeding or bleeding expansion is seen on the post-op CT scan, wait 12 hours and repeat the CT scan. When the bleeding is stable, dosing can be initiated.

If post-operative clot volume is 10 to 15 mL, rt-PA should not be given. The catheter should remain in place and open to drainage for 24-36 hours prior to removal.

Catheter adjustment/replacement: Correct catheter placement will be CT-confirmed locally and post-operative measurements will be repeated centrally by the Surgical Center. Catheter adjustments will be made at this time, if necessary. If the hematoma appears larger or the shape is altered on CT scan after the catheter is placed, the catheter may require repositioning and a post-repositioning CT scan to confirm correct placement within the clot as well as stability of clot size. Repositioning is defined as the partial removal or “pull back” of the non-optimally placed catheter. Replacement is defined as removal of the catheter and replacement with a better targeted catheter using the introducer method described above with either the same or a different trajectory of insertion. There is a one-time allowance for a new rigid cannula placement. Soft catheter placements or replacements, usually done through an existing burr hole and always done using a stylet with image guidance, do not count against this limited number of rigid cannula passes. There is no limit to the number of soft catheter placements or replacements as long as stability requirements are met. Occasionally it may be necessary to create a new burr hole/trajectory to access the clot. This will be done only after consultation with the Surgical Center. Repeat CT scan and upload DICOMs of the final catheter placement in the clot into the EDC for Surgical

Center review and approval of catheter location in residual hematoma prior to dosing.

The catheter is then tunneled subcutaneously, connected using sterile technique to a three-way stopcock and then to a closed drainage system. Level the drainage bag to zero.

If the catheter must be repositioned or replaced after dosing has begun, the procedure must be done equal to or greater than 24 hours after the most recent dose and all stability protocols must be repeated as if this were the original catheter placement.

Complete replacement of the catheter is allowed if the catheter to clot relation has been disturbed by inadvertent catheter movement or partial clot reduction. Complete replacement should be performed only once (i.e., in any subject only two, new rigid cannulas may ever be placed).

If repositioning or replacement does not correct the catheter-clot relation and the rigid cannula has already been replaced once during the trial, rt-PA administration must be stopped or not initiated and the catheter removed 24 hours later. This requirement will control the delivery of rt-PA only into space containing clot that can be lysed. The catheter may be left in place greater than 24 hours later if the catheter supports ventricular drainage as clinically required.

In addition, an unscheduled CT scan must be performed should the subject clinically deteriorate or significantly improve his or her GCS score. These additional safety provisions will keep under surveillance the most ideal time for stopping drug after clot is fully lysed.

Catheter removal. After keeping the catheter closed for the one hour following the last dose of rt-PA, the catheter should be opened and then left open to drain for 24-36 hours prior to catheter removal. The catheter must be closed at the time of removal and not open to air to avoid pneumocephalus. The catheter may be left in place greater than 24 hours later if the catheter supports ventricular drainage as clinically required.

To limit infection risk, remove the catheter at the bedside 24 to 36 hours after the last rt-PA administration, unless the catheter supports ventricular drainage as clinically required. Send the catheter tip for culture. A CT scan must be done 24 hours post catheter removal and examined for stability, new bleeding, or hemorrhage extension.

5.1.3 Experimental Drug Treatment: Drug Therapy

Prerequisite training for dosing. A neurosurgeon, neurocritical care physician, or their trained designee will perform hematoma catheter injections under standard sterile technique in the intensive care unit. Viewing a demonstration of the **catheter injection** protocol is mandatory before credentialing a center's physicians and coordinators. A training video has been produced describing the injection procedure and apparatus, sterile field techniques and the exact process for delivering the drug. The training video is available on the trial website (www.braininjuryoutcomes.com). It will be used continuously to train and retrain personnel administering the injections. A full step-by-step description of drug administration is also available in the MOP. Great care and time has been and will be expended to assure the standardization of safe drug administration. This presentation will be edited as new safety data are developed.

After placement or repositioning of any pre-dosing catheter a six-hour stabilization period is required prior to first dose to assess patient clinical status and minimize rebleeding. During this time, the neurological status of the subject will be assessed to document clinical worsening or improvement. **Surgical Center** confirmation of catheter placement, replacement, or manipulation is also required prior to initiation of dosing. Following surgery, a CT scan is to be obtained and catheter placement approved by the surgical center. This CT scan can be obtained any time prior to administering first dose. Furthermore, a period of at least 6 hours is to be observed prior to first dose to ensure subject is clinically stable.

The pharmacist will prepare the rt-PA and flush as detailed in the Pharmacy Manual, which is part of the MOP. The two prepared syringes containing the rt-PA and flush will be provided by the pharmacy to the appropriate study personnel for administration to the patient. The labels on the syringes must be compared with the patient's records to confirm identity, and to confirm correct dosage, labeling, and correct timing in the series of administration.

Dosage. The drug will be administered as a sterile solution and in a sterile manner every 8 ($\pm$ 2) hours for up to 9 doses. The total volume of injectate will equal 1.0 mg rt-PA @ 1 mg/mL plus at least 3 mL of flush or as much flush is needed for the rt-PA to clear the catheter tubing.

Dosing schedule allowances. There is a two (2) hour window on either side of the eight (8) hour dosing schedule to allow for scheduling problems, stability determination, INR correction, or any other concern the PI may have regarding giving the dose on schedule. This schedule adjustment should be used as infrequently as possible to maintain a Q8hr schedule for dosing consistency.

Holding a dose. If a dose must be held or delayed more than 10 hours to correct an INR value above 1.4 (or other coagulopathy), hold the next scheduled dose, institute corrective therapy and re-assess the INR. Once INR is corrected, dosing may be resumed keeping to the original dose count. If the INR remains > 1.4, continue corrective therapy as required and the investigator may discontinue dosing.

In the event of severe or life-threatening anaphylaxis or hypersensitivity reaction, the treatment phase would be discontinued. The patient would not be retreated with rt-PA. A description of the reaction would be added to the patient's medical record as a serious adverse event for future reference.

Dosing decision-making by CT. Before initial dosing and subsequently after every three (3) doses (daily), the investigator is required to view the most recent CT scan to measure clot size and compare to the prior day's scan in order to decide continuation or discontinuation of dosing. As clot size decreases and approaches the target reduction, the next CT may be obtained earlier than after three doses. This process allows the PI and team to confirm that: 1) remaining clot is greater than 10 mL, 2) the blood clot is in direct contact with the catheter, and 3) that the catheter is placed in the clot to be dissolved. Bone windows must be done when obtaining any CT scan in order to confirm that the catheter side ports have contact with the clot. If none of the side ports are in contact with the clot, drug should not be given. Partial contact with the clot should be reviewed by the PI on a daily basis, prior to further dosing.

Catheter repositioning during dosing. If these criteria are not met, the catheter must be repositioned. Repositioning to allow for the correct catheter to clot relationship may be performed once under direction of the investigator as needed during the dosing time of the protocol. Open the catheter to drain for 24 hours after the most recent rt-PA administration before repositioning or replacing the catheter. After repositioning obtain a repeat CT scan to confirm catheter placement and clot stability. After both are confirmed and after Surgical Center review, wait six (6) or more hours from the time of catheter repositioning and then resume dosing.

Stability determination during dosing. Stability must be demonstrated in the following ways on all CT scans: 1) no expansion of ICH greater than 5 mL as compared to the most previous CT scan, 2) no catheter tract bleed greater than 5mm, and 3) no new IVH or new expansion of IVH. If the clot is stable, dosing may resume.

Repeat CT scans will be performed earlier than every 12 to 24 hours if or when the treating physician determines that there is a sustained improvement or worsening of neurological condition (GCS motor scale score increase or decrease by more than two points for eight hours or more). Therapy will be stopped at this

time if there is any increase in hematoma volume on CT or emergence of systemic bleeding disorders. These additional safety provisions will keep under surveillance the most ideal time to stop rt-PA administration.

Intracranial pressure (if monitored), cerebral perfusion pressure, and blood pressure will be monitored before, during, and after each injection. After injection, the catheter will be closed for 1 hour to prevent drainage of the rt-PA away from the clot and to allow adequate time for drug-clot interaction. After one hour of closure, the catheter will be opened with an appropriate drainage gradient. ICP will be measured every four hours, or more frequently, as clinically indicated. A neurosurgical consult should be obtained for sustained intracranial hypertension. Sustained intracranial hypertension is defined as ICP greater than 20 mmHg for two or more consecutive hours despite maximal medical ICP management.

Documentation of dosing. In addition to documenting each dose in the EDC system, each dose administration should be documented in the Medical Administration Record of the medical record as source documentation. A progress note should also be written in the medical record as source documentation to record the date and time of each dose, the amount of rt-PA administered, the amount of flush administered, the catheter in which the drug was administered (when more than one catheter is in place), the time the catheter was closed and reopened, the ICP (if monitored), systolic blood pressure (SBP), and diastolic blood pressure (DBP) prior to the dose and prior to reopening the catheter, and the name and title of the person who administered the dose.

5.2 Handling of Study Interventions

Alteplase (recombinant human tissue-type plasminogen activator) is a sterile powder for reconstitution with Sterile Water for injection. The reconstituted preparation results in a colorless to pale yellow transparent solution containing Alteplase 1mg per ml at approximately pH 7.3. The Alteplase will be prepared in a sterile syringe labeled for investigational use. See the Alteplase Package Insert for additional product information. See MOP Chapter 19 (Pharmacy Manual) for additional instructions regarding Alteplase and flush preparation and labelling.

The following measures will be taken for storage and accountability of the investigational product:

1. The investigational product is Alteplase, which is stored refrigerated at 2-8° (36-46°F). The refrigerator temperature must be monitored and the documentation logs must be maintained for the time period that investigational product is stored at the site.

2. Accountability for each box of Alteplase will be emphasized during the training sessions.

3. Investigational product should be stored in a secure area according to local regulations. It is the responsibility of the investigator to ensure that investigational product is only dispensed to study subjects. The investigational product must be dispensed only from the official study sites by authorized personnel according to local regulations.

4. The investigational product shall be dispensed in accordance with the authorized prescriber's prescription.

5. A drug accountability form will be provided to the site. This form will be used to document all investigational product transactions (i.e., receipt of drug, dispensings, wasted doses, etc.). This documentation will be maintained at the site however, copies will be requested periodically and at the end of the study.

6. Drug accountability will be checked remotely and/or during on-site monitoring visits by review of drug accountability logs and other study documentation.

7. All unused investigational product may be discarded on site according to each site-specific policy for disposal of pharmaceutical waste. Prior to disposal of the drug, the Coordinating Center Study Pharmacist must receive a copy of the center's policy for study drug disposal (pharmaceutical waste) and documentation of the drug to be discarded.

8. The total of amount of Alteplase administered will be recorded on the source documents and case report forms.

5.3 Concomitant Interventions

5.3.1. **Required Interventions.** Intraclot administration of 1mg of rt-PA followed by 3 ml of flush every eight hours for up to nine doses.

5.3.2. **Permitted Interventions.** Study center standard of care policies may govern the use of low molecular weight, fractionated and unfractionated heparins for DVT prophylaxis during the acute treatment and follow-up periods. Heparin flushes of systemic lines are also permitted. Use of enoxaparin for DVT prophylaxis in the ICU at the usual doses of 30 mg sc Q12 h or 40 mg sc QD is permitted as long as the patient has good renal function (creatinine clearance of > 30 ml/min) or does not have an unusually low body weight (< 45 kg).

Including but not limited to NovoSeven, fresh frozen plasma, plasma concentrate and vitamin K, are permitted singly or in combination (but not required) for reversal of anticoagulation.

5.3.3. **Prohibited Interventions.** During the study period (randomization through the 12 month visit), avoid enoxaparin at therapeutic doses ≥1.0 mg/kg sc Q12 h.

After the day 30 follow up visit other antithrombotic and antiplatelet agents such as Coumadin (warfarin) and Dabigatran, glycoprotein IIb/IIIa inhibitors (eptifibatide/Integrilin, abciximab/Reopro, tirofiban/Aggrastat), ASA, clopidogrel/Plavix may be administered.

The use of Urokinase, Retevase, Desmoteplase, Tenecteplase or any other thrombolytic agent (other than the study agent) administered via any brain catheter is prohibited. Clogged catheters should be treated with normal saline flushes.

5.3.4. **Precautionary Interventions.** If any brain catheter (ICH or IVH, if present) needs to be replaced or repositioned during dosing, wait 24 h after the most recent dose to perform the procedure. A stability CT scan must then be done ≥ 6 h after all placements/repositioning to confirm correct placement, clot stability, and absence of significant blood along the catheter tract. Once these are confirmed by the site and Neurosurgical Center, dosing may restart.

If a subject experiences asymptomatic bleeding (ICH expansion < 5 cc, IVH expansion as assessed by < 2mm increase in 2 out of 3 ventricular regions, or catheter tract hemorrhage that is ≤ 5 mm in the largest diameter), continue the dosing and CT schedule. If the bleeding is larger than these thresholds, with or without mass effect, the next scheduled dose is held and a repeat CT scan is done ≥ 24 h after the previous dose. If the ICH, IVH, and catheter tract hemorrhage are stable (i.e., has not further grown by > 5 mm), then dosing may restart.

Particular caution needs to be observed with renal dialysis patients receiving rt-PA. Because this group of patients can experience wide variations in blood pressure with dialysis attendant cardiac volume changes, attention to long-term and intra-procedure blood pressure control is important. Similarly, attention to regional anti-coagulation management is important.

Consider administering platelets to eligible patients who are on antiplatelet therapy (Plavix, aspirin, etc.) at the time of symptom onset. Platelet counts should be closely monitored in this population.

5.4 Adherence Assessment

Protocol adherence will be determined by review of data recorded on the case report forms that has been verified through comparison with the medical record and other source documentation. Compliance and treatment fidelity will be reported overall and by center to the DSMB at each scheduled review session. Study centers demonstrating poor protocol compliance will be retrained and, if necessary, replaced.

6 CLINICAL AND LABORATORY EVALUATIONS

6.1 Schedule of Evaluations

	Screening / Baseline	Day 1 (day of randomization)	Day 2	Day 3	Days* 4 - 6	Day 7	Day 30 ± 7 days	Day 90 ± 7 days	Day 180 ± 14 days	Day 270 ± 14 days	Day 365 ± 14 days
Diagnostic CT	X										
Informed consent	X										
Stability CT (6 hours after diagnostic CT)	X										
Pregnancy test	X										
Medical History/ Review of Systems	X										
CTA (MRA)/MRI	X					Day 7-10 ± 1 day X (MRI)					
Image-Guided Catheter Placement + Aspiration (Surgical Group Only)		X									
Post catheter placement CT scan		X									
rt-PA admin. (Surgical Group Only; Q8h up to 9 doses)		X	X	X							
MTI-M3 specimen collection		X	X	X	X						
Daily CT Scan		X§	X	X	X (Day 4)		X†		X†		
		obtain a CT 24 h post catheter removal on any of these days									
Vital Signs		X	X	X	X						
Neurocheck		X	X	X	X						
Lab Assessments*	X	X	X	X	X						
Concomitant treatments	X	X	X	X	X	X	X	X	X	X	X
NIHSS	X					X	X		X		X
Barthel Index	X						X	X	X	X	X
Modified Rankin Scale	Historic						X	X	X	X	X
Stroke Impact Scale							X		X		X
GOS-E Scale							X		X		X
Mini-Mental Exam							X		X		X
Euro-Quol-5D Scale							X	X	X	X	X
PBSI							X		X		X
Personal Health Utility Assessment Interview									X		
CES-D									X		

*Assessments should be performed daily through Day 6 regardless of treatment assignment.
†An MRI is an acceptable substitute for this CT scan with Coordinating Center approval.
§ Subjects enrolled to medical management: The Day 1 CT scan does not need to be repeated if enrollment occurs on the same calendar day as the stability CT scan (done 6h post the diagnostic CT scan).

6.2 Timing of Evaluations

6.2.1 Screening/Baseline.

Screening/Baseline is the start of the screening process and begins when the study team is notified of a potentially eligible subject. If any of the assessments required to determine eligibility (stability CT scan, pregnancy test, serum lab assessments) are not part of routine patient management, then these assessments will be listed in the consent form as trial-related procedures and informed consent will be obtained prior to determining eligibility for the trial. The prospective subject will be informed as part of the consent discussion that the screening procedures will determine eligibility for the trial and that signing the consent form does not constitute enrollment into the trial.

6.2.2 Day 1 through Day 6

The acute phase of the protocol is defined as day one (day of randomization) through day six post randomization. All subjects randomized to the surgical intervention plus rt-PA will receive up to nine doses of intracatheter injections of rt-PA. Dosing will be discontinued prior to nine doses if an endpoint is satisfied.

Randomization should occur as close as possible to the time that all eligibility criteria are met. If randomization is postponed to accommodate scheduling (i.e., it is preferable to wait until 6 am to randomize instead of midnight), obtain a CT scan to re-confirm stability of the ICH and re-confirm blood pressure stability prior to randomization. For those subjects randomized to surgery (MIS+rt-PA) the surgical procedure should be initiated as close as possible to the time of randomization.

6.2.3 Day 7 through Day 365

The follow-up phase of the protocol begins on day 7 and continues through the 12-month follow-up visit.

6.2.4 Intervention Discontinuation Evaluations

Subjects withdrawn early from treatment or who withdraw consent or are lost to follow-up will not be replaced. If a subject is withdrawn early from treatment due to a clinical safety endpoint, we expect standard clinical judgment to be applied to continue to monitor the subject until resolution of the event including but not limited to repeat CT scans and repeat laboratory assessments.

Discontinuation of drug administration and catheter removal. rt-PA injections will continue as defined by the protocol until an endpoint of clot lysis is reached (i.e., clot is reduced to 10-15 mL or nine doses are administered, whichever

comes first) or an adverse treatment endpoint occurs, such as symptomatic hemorrhage. ICH clot resolution of 10-15 mL will be estimated by comparing the daily dosing CT scans and performing the ABC/2 measurement on sequential scans. A CT scan must be done one day after the catheter is removed to monitor for new bleeding or bleeding extension. After last dose the catheter will be closed for one hour and then reopened to drain for 24 hours to allow for complete removal of rt-PA. The catheter must be closed at the time of removal to avoid pneumocephalus.

A subject will have rt-PA administrations discontinued for any of the following reasons:

1) The ICH volume is reduced to less than or equal to 10 mL (surgical endpoint).
2) The subject receives nine doses of rt-PA (surgical endpoint).
3) Clinically significant rebleeding (treatment failure).
4) Uncontrolled coagulopathy defined as INR > 1.4 (treatment termination).
5) In the investigator's judgment, withdrawal from the trial would be in the patient's best interest (treatment failure).
6) The patient withdraws consent.

6.2.5 On Study/Off-Intervention Evaluations

The follow-up phase of the protocol begins on day seven and continues through the 12-month follow-up visit. All subjects will be required to return for a follow-up clinic visit at months one, six, and 12, with a CT scan to be done at the one and 6 month visit. A telephone follow-up visit will occur at months three and nine. Daily monitoring of all adverse events will continue until day six. This includes monitoring of additional medications used, additional procedures and ICU care required. Serious adverse events will be monitored throughout the initial hospitalization. Serious adverse events, neurological adverse events, and total time at home (i.e., excluding hospital re-admissions and admission to rehabilitation facilities) will be recorded at all subsequent follow-up visits.

Subjects withdrawn early from the treatment protocol are asked to return to clinic for all scheduled follow-up assessments including the one, three (telephone contact), six, nine (telephone contact) and 12 month visits.

6.2.6 Final On-Study Evaluations

At the subject's final visit to occur at approximately 12 months post ictus, the following will be done: NIHSS, Barthel Index, videotaped modified Rankin Scale, extended GOS, Stroke Impact Scale, Mini-Mental State Exam, EQ-5D, and PBSI. Also at this time the subject will be asked about any new neurological adverse events or any serious adverse events that may have occurred since the nine-month telephone contact. The subject will also be specifically asked about

any neurosurgical procedures. All serious and non-serious adverse events that occurred prior to the 12 month visit but remain documented as "ongoing" will be confirmed as "ongoing" or documented as "resolved" and a resolution date recorded. The examiner will document if the subject was prescribed and is compliant with any blood pressure medications prior to the 12 month visit and document if any of the following have occurred since the nine-month telephone contact: death, new brain bleeding, brain bleeding extension, and/or cerebral infection. The subject will be instructed that this is the final visit.

6.2.7 Pregnancy

Pregnant women are ineligible to participate in the trial. Women who become pregnant during the follow-up period will be followed through the 12 month visit to document clinical and functional outcome but no CT scans will be done.

6.3 Special Instructions and Definitions of Evaluations

6.3.1 **Diagnostic CT:** This CT scan is the initial CT used to diagnose the ICH and is done per standard medical care upon presentation to the Emergency Department. If this scan is done at an outside hospital prior to transfer to the enrolling center, the outside hospital scan must be obtained and uploaded to the EDC. CT angiogram or routine angiogram with evaluation for "spot sign" is encouraged to complete the evaluation for aneurysm, AVM, or other malformations. If this imaging is not done, the rationale must be documented in the EDC system. The dCT scan will be used to calculate the ICH and as the start time for the 72 hour surgery initiation window. ICH size less than 30 mL on this scan does not exclude the patient from participation. The patient should be followed until the enrollment window closes to monitor for ICH expansion. The dCT scan will be compared with the first stability CT scan to determine if the hemorrhage continues to expand or if stability has been achieved (ICH size does not increase by ≥ 5 mL). A copy of the dCT DICOMs will be uploaded to the EDC prior to randomization. The Reading Center will centrally review this scan if requested to confirm eligibility and to measure ICH (and IVH if present) clot volume for efficacy analysis.

At each study center ICH volume will be determined in a standardized manner. Instructions for calculating ICH volume are included in the MOP.

The size of the ICH and the IVH along with the date and time of the dCT scan should be documented in the medical record as source documentation.

6.3.2 **Informed consent:** Consent forms must be reviewed by the CCC for completeness and accuracy prior to submission to local study center IRB/Ethics Committees. This review must occur after each time the document is modified.

The informed consent process can begin at any time during the screening process but must be obtained prior to randomization. A signature on the consent form does not translate into enrollment in the study. Only after all inclusion/exclusion criteria have been met and informed consent has been signed can a patient be randomized into the study.

Informed consent must be obtained from the patient, or if the patient is aphasic, confused, or obtunded, the legal representative of that patient. Patients will not be treated if consent cannot be obtained from a competent patient or from their legal representative.

The study center will document the informed consent process and the signing of the consent form in a written progress note, place a signed copy of the consent form in the hospital medical chart, and keep the signed original consent form in the study subject file. A signed copy must be given to the subject as well. The study monitor will review and confirm the signed consent form while reviewing subject data collection forms and/or during on-site monitoring visits.

6.3.3 **Stability CT** (six or more hours after dCT): See page 23, 2. Stability CT scan. If this CT is not done per standard medical care at a participating study center, informed consent must be obtained prior to ordering the CT. All CT scans done after the dCT and prior to randomization are considered stability CT scans. All stability CT scans must be reviewed by a radiologist or an investigator and compared to the Diagnostic or most previous CT to confirm that the ICH (and IVH if present) clot is stable. A DICOM formatted copy of the final stability CT scan used for randomization will be uploaded to the EDC prior to randomization. All other stability CT scans done after the dCT and prior to randomization will be uploaded to the EDC prior to day 7. The Surgical Center will review the images as outlined on page 29. The Reading Center will centrally review this scan/these scans to measure stability of ICH, IVH, and catheter tract clot volume.

The size of the ICH (IVH, if present) along with the date and time of all stability CT scans should be documented in the medical record as source documentation.

6.3.4 **Pregnancy test:** Female patients of childbearing ability (i.e., of childbearing age and not surgically sterilized) must have a negative urine or serum pregnancy test to be eligible. If this test is not done per standard medical care at a participating study center, informed consent must be obtained prior to ordering the test.

The date and time of the pregnancy test and the result should be documented in the medical record as source documentation.

6.3.5 **Medical history/Review of systems:** The medical/treatment history must be documented as part of the screening process to rule out exclusion criteria (i.e., serious concurrent illness, clotting disorder, known risk for embolization, etc.).

Medical history obtained for data collection purposes may be recorded in the medical record as source documentation and then transcribed to the VISION EDC system or can be recorded directly on the eCRF to document discussions with the patient, family, and/or health care team not otherwise collected in the medical record.

6.3.6 **CTA (or MRA) and MRI:** See section 4.3.1: Screening Procedures, 3. Imaging to rule out underlying pathology on page 24 above. Repeat imaging (MRI) will be done on day 7-10 (± 1 day), unless contraindicated, to assess cerebral edema, cerebral infarction, and other clinical sequelae.

6.3.6 **Image-Guided Catheter Placement + Aspiration (Surgical Group Only).** See section 5.1.2: Experimental Intervention: MISTIE-Surgery, above.

6.3.7 **Post catheter placement CT scan:** A CT scan will be done after the MIS procedure as described in section 5.1.2: Experimental Intervention: MISTIE-Surgery, above.

6.3.8 **rt-PA administration:** See section 5.1.3: Experimental Drug Treatment: Drug Therapy, above.

6.3.9 **Daily CT scan:** A CT scan is required daily on days one through four for both surgical and medical subjects. For surgical subjects, a CT scan is repeated one day (approximately 24 hrs) post catheter removal. During dosing, all patients must receive a minimum of one scan per day, preferably in the morning, but at least after every three doses are administered. This CT scan will monitor for clot lysis and asymptomatic bleeding and will be evaluated by the investigator prior to the next administration of rt-PA. This does not represent an increase in the total number of scans requested; rather, it reflects two data collection goals: 1) to match drug administration times with independent assessment of safety and efficacy data points and to provide additional safety precautions during dosing; and 2) to collect the primary surrogate outcome measure on a fixed daily schedule for optimal measurement of the rate of clot resolution.

The catheter tract must be reviewed on the daily CT scan to determine if there is a new onset or expansion of catheter tract hemorrhage.

The date and time of all daily and unscheduled CT scans should be recorded in the medical record as source documentation. We will also record and analyze all CT scans ordered during the acute hospitalization.

6.3.10 **Vital Signs:** Monitoring of vital signs includes documentation of blood pressure and ICP (if monitored).. Vital signs are to be collected once every six hours beginning at randomization through day six.

6.3.11 **Therapy Intensity Levels (TILs):** In addition, therapy intensity levels will be documented at Baseline and day seven. The TILs will be used to document the management intensity of reversal of anticoagulation, Glasgow coma score, blood pressure, temperature, blood glucose, ICP, and cerebral herniation.

All vital signs and TIL data must be recorded in the medical record, or as ICU monitor print outs as source documentation.

6.3.12 **Neurocheck:** GCS may be assessed clinically as frequently as every hour. For the purposes of neuromonitoring, GCS will be recorded once every six hours to assess for neuroworsening or clinical improvement. An unscheduled CT scan should be done if the subject improves or worsens by more than two points on the GCS motor scale that is sustained for at least eight hours, or sooner if clinically indicated.

6.3.13 **Lab Assessments:** The results of daily white blood cell count, serum glucose, platelet count, aPTT, and INR will be reviewed and recorded in the EDC. These lab assessments will be monitored through day six. The following lab assessments must be done and reviewed to screen the patient for eligibility: platelet count, INR, and, if applicable, urine or serum pregnancy test. The INR must remain $\leq$ 1.4 during dosing.

All lab results and sampling dates and times must be recorded in the medical record as source documentation.

6.3.14 **Concomitant treatments:** All concomitant medications administered that are inclusive of the drug classes of interest and procedures performed (randomization through day 6) will be recorded on the eCRF. Drug classes of interest include but are not limited to: anti-hypertensives, sedatives, hypnotics, hematologic modifiers, antiplatelet and anticoagulant medications, antibiotics and any other medication used to treat a neurological adverse event or any serious adverse event. Medications used to treat a neurological adverse event or any serious adverse event will be recorded on the eCRF through day 365.

6.3.15 **NIHSS.** The NIHSS should be done by a certified examiner as close to the time of randomization as possible, at day seven, and again at months one, six, and 12.

The NIHSS results may be recorded directly on the electronic case report form.

6.3.16 **Barthel Index.** A historical Barthel Index score should be obtained to assess the patient's level of functioning, prior to symptom onset and will be used in a comparison with scores obtained at one, three, six, nine, and 12 months.

The Barthel Index items and total score may be recorded directly on the electronic case report forms.

6.3.17 **Modified Rankin Scale.** A historical modified Rankin Scale score should be obtained as part of the screening procedures. The patient must have a mRS score of 0 or 1 to be eligible for the study. This historical score is based on the patient's level of functioning prior to the onset of symptoms and will be used in a comparison with scores obtained at one, three, six, nine, and 12 months. The one, six, and 12 month evaluations will be done by a certified examiner and videotaped with digital images sent to the Outcome CCC at the Western Infirmary in Glasgow, UK. A sample consent form for videotaping an interview with a proxy caregiver can be found in Appendix 4.

The historical modified Rankin score may be recorded directly on the electronic case report forms. The Outcome Coordinating Center will adjudicate all follow-up mRS scores.

6.3.18 **Stroke Impact Scale (SIS).** A SIS score should be obtained as part of the follow-up procedures at one, six, and 12 months. If the subject has a Mini-Mental exam score of 18-30, you should attempt to interview the subject. If the subject is unable to complete the interview or has a Mini-Mental score < 18, then an appropriate caregiver should be interviewed to complete the SIS.

The SIS must be recorded on the bedside worksheets and then entered into the EDC. The bedside worksheets are required source documentation.

6.3.19 **Extended Glasgow Outcome Scale (GOSE).** A GOSE score should be obtained as part of the follow-up procedures at one, six, and 12 months. If the subject has a Mini-Mental exam score of 18-30, you should attempt to interview the subject. If the subject is unable to complete the interview or has a Mini-Mental score < 18, then an appropriate caregiver should be interviewed to complete the GOSE. A GOS score will be computed by the Statistical Center from the GOSE scale.

The GOSE must be recorded on the bedside worksheets and then entered into the EDC. The bedside worksheets are required source documentation.

6.3.20 **Mini-Mental Exam.** A Mini-Mental exam will be done as part of the follow-up procedures to determine the subject's ability to complete the GOSE and SIS interviews. If a subject has a Mini-Mental score of 18-30, subject interview will be attempted. If the subject is unable to complete the interview or has a Mini-Mental score < 18, then an appropriate caregiver should be interviewed to complete the GOSE and the SIS at one, six, and 12 months.

The Mini-Mental Exam may be recorded directly on the electronic case report forms.

6.3.21 **Euro-Quol-5D.** An EQ-5D score should be obtained as part of the follow-up procedures at one, three, six, nine, and 12 months.

The EQ-5D must be recorded on the bedside worksheets and then entered into the EDC. The bedside worksheets are required source documentation.

6.3.22 **Preference-Based Stroke Index.** A PBSI score should be obtained as part of the follow-up procedures at one, six, and 12 months. If the subject has a Mini-Mental exam score of 18-30, you should attempt to interview the subject. If the subject is unable to complete the interview or has a Mini-Mental score < 18, then an appropriate caregiver should be interviewed to complete the PBSI.

The PBSI must be recorded on the bedside worksheets and then entered into the EDC. The bedside worksheets are required source documentation.

6.3.23 **Personal Health Utility Assessment Interview.** A Personal Health Utility Assessment Interview will be done as part of the follow-up procedures at six months.

The Interview must be recorded on the bedside worksheets and then entered into the EDC. The bedside worksheets are required source documentation.

6.3.24 **Center for Epidemiological Studies – Depression screen (CES-D).** A CES-D screen will be done as part of the follow-up procedures at six months.

The CES-D must be recorded on the bedside worksheets and then entered into the EDC. The bedside worksheets are required source documentation.

7 MANAGEMENT OF ADVERSE EXPERIENCES

In the event of an adverse event, the first concern will be for the safety of the subject. Investigators are required to report to the CCC any serious adverse event and any adverse event which is assessed by the investigator to be possibly, probably, or definitely related to the surgical procedure or rt-PA. All events meeting these criteria will be reported for the time period beginning with randomization through the protocol-defined follow-up. Serious criteria, definitions, and guidance for reporting follow in section 10.4: Adverse Experience Reporting.

Management of recurrent bleeding. Best care criteria defined by the American Heart Association (AHA) guidelines for management of ICH will be the standards of care for all general medical care in this protocol. Specifically, the guidelines are (1) Stroke Council special writing group guidelines for the management of ICH,[11] (2) European Stroke Initiative (EUSI) Guidelines for the management of intracranial hemorrhage,[81] and (3) the AANS guidelines for management of elevated intracranial pressure.[82] Adverse

events will be managed similarly by employing these guidelines. Specific management for new intracranial bleeding does not exist. Each instance is managed by the care team as required to preserve life and function. Management may include blood pressure reduction, use of platelets and clotting factors, use of prothrombotic agents, and use of a surgical procedure. The management of each adverse event will be recorded and may be reviewed by the Safety Event Committee.

Management of brain infection. Routine antibiotic management of symptomatic brain infection (bacterial or non-bacterial) will be performed according to accepted principles of infection care. The selection of an antibiotic on the basis of culture and sensitivity data will be the primary means of management. Removal of any infected hardware and the subsequent adjustment of antibiotic on the basis of response to therapy will be applied to all patients.

The occurrence and management of all brain infections, with and without positive CSF cultures, will be recorded and reviewed reported by the Safety Event Committee so that an independent assessment of clinical significance may be made as necessary.

We consider both bacterial ventriculitis and bacterial meningitis compulsory SAEs and treat both the same. This is the most conservative safety profile. We provide separate SAE codes for a clinical determination between ventriculitis and meningitis and bacterial and non-bacterial, to accommodate investigator diagnostic classifications.

Procedures for modification. Injections of rt-PA will be discontinued for all symptomatic hemorrhage occurrences. No other modification of "best care" is anticipated for symptomatic hemorrhage. All care associated with this event will be recorded on the SAE eCRF. Injection of rt-PA will not be discontinued for infection. The same adverse event reporting will be employed.

8 CRITERIA FOR INTERVENTION DISCONTINUATION

rt-PA administration will be discontinued for any of the following reasons:

1. Dosing will be discontinued when the ICH clot volume is reduced to less than or equal to 10 mL, or the subject receives 9 doses of rt-PA (treatment endpoint), whichever occurs first.
2. Dosing will not be started if the ICH clot volume is reduced to less than or equal to 10 mL by clot aspiration alone during catheter placement (surgical endpoint).
3. The subject experiences a clinically significant bleeding event (local or systemic) (treatment failure).
4. In the investigator's judgment, withdrawal from the trial would be in the subject's best interest (treatment failure).
5. The subject or legal representative withdraws consent.

All subjects will be followed by the site team through day 365 (reasons 1 to 5) as part of the outcome assessment for the intervention. The use of the phone and mail will be the main means of maintaining contact after discharge. The subject or legal representative has the right to withdraw consent. At the time of consent withdrawal, the site personnel will discuss with the subject or legal representative the level of their consent withdrawal to determine if they are willing to discontinue study treatment but agree to in-person and by phone follow up visits, disagree to follow up visits but agree to phone contact to determine vital status, or no further contact at all.

9 STATISTICAL CONSIDERATIONS

9.1. Statistical Analysis Plan

Primary Efficacy. The primary objective of this multicenter, randomized, controlled, Phase III clinical trial is to determine the efficacy of intra-clot catheter placement and aspiration of hematoma contents, followed by gentle clot irrigation with low dose recombinant tissue plasminogen activator (rt-PA), referred to as Minimally-Invasive Surgery plus rt-PA (MIS+rt-PA). The primary hypothesis of this trial is that the MIS+rt-PA approach to intracerebral hemorrhage (ICH) management will result in more patients having overall better functional outcome at 180 days, as defined by the modified Rankin Scale (mRS)1 in the range 0-3, when compared to patients managed with standard aggressive medical treatment. Although the mRS includes a category for death, and hence the primary analysis incorporates mortality outcomes, mortality as a separate outcome will also be examined. An intent-to-treat (ITT) paradigm for the analyses will be incorporated. The proposed trial would enroll a total of 500 patients, 250 randomized to each treatment arm (MIS+rt-PA and medical management). The power to detect the effect size of 13% across all clot locations with this sample size is 88%. The power to detect the conservatively estimated effect size of 11% in this patient population is 81%.

Efficacy Measures Summary.

- **Primary measure:**

Dichotomized adjudicated mRS 0-3 vs. 4-6 at 180 days post-stroke

- **Secondary measures:**

1. Dichotomized adjudicated mRS at 365 days post-stroke 0-3 vs. 4-6 and 0-2 vs. 3-6
2. Ordinal adjudicated mRS (0 – 6) at 180 days post-stroke
3. Mortality and Safety Events at 30 days post-randomization, including procedure-related mortality, symptomatic bleeding rate, and infection rate
4. Mortality at 180 days post stroke
5. Functional Status: NIHSS, Barthel Index, GOS, extended GOS, MMSE at 180 days post-stroke
6. Type and intensity of ICU management: ICU days, hospital days, patient disposition at 180 and 365 days post-stroke

7. Quality of life: SIS, EQ-5D, PBSI, Personal Health Utility Assessment Interview, days to return to home; patient disposition at 180 and 365 days post-stroke including proportion of days in long-term care facilities
8. Cost: amount of billed and reimbursed

In addition to the primary and secondary measures, there are a number of related and intermediate surrogate outcomes of interest, including Glasgow Coma Score (GCS), Graeb Scale, clot lysis rate and final clot size reduction.

Safety Measures. Interim safety analyses will be prepared for the external Data Safety and Monitoring Board (DSMB) on a pre-arranged schedule (such as semi-annual or after enrollment of a fixed number of subjects) to evaluate efficacy and safety. Safety measures include: monitoring the incidence of symptomatic and asymptomatic intracranial bleeding events (i.e., hemorrhage extension, new hemorrhage, catheter-tract hemorrhage) through daily computerized tomography (CT) scans for the first four days after randomization and then repeated one day (approximately 24 hours) after ICH catheter removal, and monitoring the incidence of confirmed and suspected infection and in-hospital mortality.

Safety Monitoring and Recruitment Suspension Rules. Safety events above pre-specified thresholds will trigger "a suspend recruitment and review "by the DSMB to investigate the presumed cause and impact of these events. Such events are initially reviewed by a Data Coordinating Center (DCC) staff member to determine if an event threshold has been reached at which time the study investigators and the DSMB will be notified. The thresholds triggering such a review are: 30-day mortality > 60%, a MIS+rt-PA related symptomatic bleeding rate > 25% (events identified within the first 30 days and assessed as occurring during active treatment or during the 72 hours after treatment will be monitored), first week operative death rate > 10%, and a procedure-related infection rate > 15% (over the initial 30 days). If any of these SAEs are attributable to the intervention (catheter insertion or manipulation, or rt-PA injection), the study will be suspended for a complete safety and efficacy review.

Efficacy Monitoring and Recruitment Suspension Rules. Interim analyses for safety will occur after 125, 250, 375 and 500 subjects are enrolled. One interim look at efficacy and futility will occur after 375 subjects are enrolled. These analyses will be based on 'Brien-Fleming' stopping boundaries for efficacy and more aggressive (i.e., more likely to stop if there is no early signal of benefit) stopping boundaries for futility.(Jennison C, Group sequential methods with applications to clinical trials. Boca Raton, FL. Chapman & Hall/CRC Press, 2000.)

9.1.1. Randomization. Subjects will be randomized to MIS+rt-PA surgery: medical management using a covariate-adaptive design similar to that used for the CLEAR III trial. The goal of this randomization scheme is to obtain an improved balance across study arms in the number of subjects with certain pre-randomization variables that are strongly predictive of the functional outcomes

(mRS) at 180 days post-stroke. These variables include pre-randomization clot volume, and baseline severity of impairment as measured by GCS. The design of Pocock and Simon[83] will be used, which increases a newly enrolled subject's probability of being assigned to the study arm that improves the overall balance in these important prognostic factors.[84] Briefly, prior to randomization for each incoming patient, the imbalance between treatment arms will be determined using the accumulating available data on clot volume and GCS. The patient will then be randomized with high probability (e.g. 'weighted coin toss') to the treatment arm that lowers the imbalance. Patients at any given site will not be considered for adaptive randomization until the site has enrolled two patients into each treatment arm by within-site block randomization. This randomization scheme will be implemented using software included in the MISTIE III data base similar to what has been successfully implemented in the CLEAR III trial.

9.1.2. Blinding. The examiner performing the 30, 180, and 365 day follow-up assessments will video tape the mRS interview assessments and upload the video to Glasgow where trained reviewers will classify the interview objectively without knowledge of the examiner's score, patient name, or of the treatment details including treatment intensity.

9.1.3. Target Population and Study Samples

i. Target Population. The proposed trial design calls for the enrollment patients with ICH $\geq$ 30 mL and not requiring extraventricular drainage (EVD) for the management of obstructive hydrocephalus. The proposed plan includes patients with ICH clots in all locations of the brain.

ii. Intent-to-Treat Sample. As the primary analysis, all efficacy and safety outcome measures are analyzed under the ITT. Under this principle, the evaluable sample includes all subjects who are randomized and receive at least one dose of Alteplase and each subject is analyzed according to the treatment group to which they were assigned at the time of randomization.

iii. Safety Analysis Sample. All randomized subjects are included in the safety analysis sample.

iv. Per-Protocol Sample. The potential for cross-overs in this study is minimal; none occurred in the phase II trial. However, in the case of cross-overs, a per-protocol sample will be constructed and examined in which treatment-as-received is analyzed. Given that clot resolution is a potentially treatment-related post-randomization variable, this covariate will be examined as an important potential mediator of final outcomes as part of a per-protocol analysis.

9.1.4. Overall Methodology Description. Statistical analyses will be performed on data that have been quality-assured through the Data Coordinating Center's (DCC)

protocols and monitoring reviews and have been exported by the DCC directly from the MISTIE III data base (Section D.3.e - DCC grant). The following analysis procedures may be applied to blinded (no treatment arm designation, for study investigators and staff), partially unblinded (treatment designation only as A and B, for DSMB and at interim analyses), and unblinded (full treatment designation, for final analyses) data, by DCC staff having the appropriate role permissions.

9.1.5. Patient Disposition. Summary statistics will be presented for the analysis sets and subgroups, the patients who completed the study, the patients who discontinued early from the study, and the reasons for early discontinuation including bleeding, loss to follow up, patient withdrawal or refusal, other complicating disease, error, or other reasons.

9.1.6. Baseline Characteristics.

i. **Demographics.** Summary statistics will be presented for age (years), gender, ethnicity, presentation center, hypertension history, diabetes, coumadin use, antiplatelet drug use.

ii. **Medical History.** Summary statistics will be presented for medical history, as well as presentation temperature, pulse, systolic blood pressure, and diastolic blood pressure. Presenting NIHSS, intracranial pressure (ICP), ICH volume, IVH volume, location of bleed and IVH score will be provided.

9.1.7. Efficacy Analyses

9.1.7.a. Analysis by MISTIE III Specific Aims

Primary Aims

Hypothesis 1a: Minimally invasive surgery (MIS) plus recombinant tissue plasminogen activator (rt-PA) for 3 days for the treatment of ICH improves functional outcome at 180 days as measured by the adjudicated mRS 0-3 by an estimated 13% increase as compared to the medically-managed patients. The null hypothesis will be a test of no improvement of MIS+rt-PA over medical management. Below is a description of the procedure for computing estimators of the treatment difference in probability of mRS 0-3 at 180 days post-stroke, for the study's population.

Analysis 1a: To evaluate this null hypothesis in the context of the covariate-adaptive randomization scheme, the model in equation (1) will be used, where Trt is 1 for MIS=rt-PA patients and 0 for medically-managed patients, ICH is pre-randomization ICH clot volume, GCS, Age, Diabetes and CVD (cardiovascular disease) are at randomization.

$$(\text{logit}(Prob[mRS \leq 3])) = \beta_0 + \beta_1 Trt + \beta_2 ICH + \beta_3 GCS + \beta_4 Age + \beta_5 Diabetes + \beta_6 CVD \quad (1)$$

The standardized estimator of the marginal treatment effect, in the form of RD, will then be computed. The advantage of this estimator, compared to the estimator based on a simple test of proportions using a z-test without covariates, is that it can lead to greater power for hypothesis testing.[85]

The estimator in this approach is standardized to the overall distribution of covariates in equation (1) across the two treatment arms. Under this framework, the probability of a good outcome is defined under two scenarios for each patient: if assigned to MIS+rt-PA treatment vs. if assigned to the standard medical treatment arm (i.e. the counter-factual probabilities). The counter-factual probabilities based on model 1 are presented below as equations (2) and (3):

$$\mu_{1i} = logit^{-1}(\hat{\beta}_0 + \hat{\beta}_1 + \hat{\beta}_2 ICH_i + \hat{\beta}_3 GCS_i + \hat{\beta}_4 Age_i + \hat{\beta}_5 Diabetes_i + \hat{\beta}_6 CVD_i) \quad (2)$$

$$\mu_{0i} = logit^{-1}(\hat{\beta}_0 + \hat{\beta}_2 ICH_i + \hat{\beta}_3 GCSi + \hat{\beta}_4 Age_i + \hat{\beta}_5 Diabetes_i + \hat{\beta}_6 CVD_i) \quad (3)$$

In equation (2), all patients are 'forced' into the MIS+rt-PA group (i.e., Trt=1) and $\hat{\beta}_1$ estimates the treatment effect, while in equation (3), all patients are 'forced' into medical management (i.e., Trt=0) and $\hat{\beta}_1$ drops out. The standardized estimator of the average treatment effect, using (2) and (3) above, is obtained by first computing the average of the patient-specific counter-factual probabilities of a good outcome under assignment to each treatment arm, across all patients. Next the difference is taken between this average under assignment to the MIS+rt-PA arm, and this average under assignment to the medical management arm.

To derive the average treatment effect, the patient-specific probabilities are averaged across patients. The average treatment effect on the log odds ratio scale can be formulated according to equation (4).

$$RD = \frac{1}{n}(\sum_{i=1}^{n} \hat{\mu}_{1i} - \sum_{i=1}^{n} \hat{\mu}_{0i}) \quad (4)$$

It has been shown that this estimator is consistent even if the model in equation (1) is mis-specified.[86] To test the null hypothesis, a Wald statistic will be computed that is equal to the above estimator of the ITT average treatment effect between assignment to MIS+rt-PA versus assignment to medical management (on the risk difference scale) divided by its estimated standard error. Rejection of this null hypothesis will be based on group sequential thresholds (Table 1 below).

In subsequent analyses the model in equation (1) will be modified to include another baseline severity measure, such as NIHSS. In addition, a model with IVH volume will be explored. The models will be compared using Akaike Information Criterion (AIC) to assess the best combinations of predictors. The discriminatory ability of the models will be compared using cross-validated area under the Receiver Operating Characteristic (ROC) curve.

Hypothesis 1b: Early use of MIS plus rt-PA for 3 days for the treatment of ICH is as safe as medically-managed ICH, as measured by rates of procedure-related mortality, rebleeding, and infection within 30 days post-randomization. The null hypothesis is: *Early use of MIS+rt-PA is worse than medical management for a specific safety measure.*

Analyses 1b: The number of severe adverse events (SAEs) will be tabulated by SAE type at each follow-up time point for the total population as well as by treatment group, with particular emphasis placed on group differences at 30 days post-randomization. Any treatment group differences in SAEs enumerated for interim analyses will remain blinded to investigators associated with this study. Partially un-blinded results (e.g. group 'A' versus 'B' where the labels 'A' and 'B' are randomly assigned to the surgical and medically-managed groups) will be made available only during the closed sessions of DSMB meetings.

Methods of equivalence[87] will be used to statistically determine if the proportions of a particular type of SAE are equal among both treatment groups in order to test the hypothesis that MIS plus rt-PA is safe. Equivalence testing deems the rates of any event to be equivalent between populations as long as the group difference in this rate is statistically within some pre-specified tolerance. For this trial, the tolerance value will be defined as 10% for the primary adverse events of 30-day mortality, rebleeding, and infection, and 5% for first-week operative mortality. Thus, the rates of adverse events seen in the MIS+rt-PA group can be higher than those in the medically-managed group, and yet still be considered equivalent as long as the upper one-sided 95% confidence intervals on rate differences are $\leq 10\%$, or $\underline{\leq} 5\%$, depending on the safety measure.

Specific Aim 2.

Hypothesis 2a: EOT clot volume and/or percent EOT ICH reduction are related to mRS functional outcome regardless of treatment. The null hypothesis is: *EOT clot volume and/or percent EOT ICH reduction trajectories has/have no effect on functional outcome regardless of treatment.*

Analysis 2a: This hypothesis investigates the relationship between the magnitude of clot resolution and functional outcome as measured by dichotomized mRS scores (i.e., mRS 0-3 vs. 4-6) at 180 days post-stroke. This is an important component of analysis that has been missing from other surgical trials and will provide for a very stringent test of volume-reduction hypothesis. The goal of this analysis is to test the importance, or lack thereof, for volume-reduction goals in all study patients. This will be best implemented by considering the relationship between clot resolution and functional outcome, as measured by mRS, irrespective of treatment assignment.

The analysis for this aim will proceed in two phases. In the first phase a model for clot resolution over time will be developed. In the second phase, predicted trajectories from phase one will be used to assess the relationship between the magnitude of clot resolution and "good" outcome at 180 days. Generalized Linear Mixed Models (GLMMs) will be

used[88,89] to estimate patient-specific clot resolution over time. These models will include a random intercept for each patient to represent unobserved factors that are common to ICH volumes for a given patient and a random slope to represent the heterogeneity of clot-resolution trajectories over time across patients as shown in equation 5.

$$ICH_{ij} = \beta_{0i} + \beta_{1i} Time_{ij} + \varepsilon_{ij}\ , \quad (5)$$
$$\beta_{0i} = \gamma_{00} + u_{0i}$$
$$\beta_{1i} = \gamma_{10} + u_{1i}$$
$$\text{where } \varepsilon_{ij} \sim N(0, \sigma^2) \text{ and } \begin{pmatrix} u_{0i} \\ u_{1i} \end{pmatrix} \sim N\left[\begin{pmatrix} 0 \\ 0 \end{pmatrix}, \begin{pmatrix} \tau_0^2 & \tau_{01} \\ \tau_{01} & \tau_1^2 \end{pmatrix}\right]$$

Several extensions to the "base model" described above will be considered by changing the assumptions about the random effects and their variance-covariance matrix structure, including models a) without random intercept, b). without random slope, c) with heteroscedastic within person residual errors, d) with grouped covariance structure by study arm, where the variance-covariance matrix of random effects becomes a block-diagonal matrix, where one block represents the variances and covariances of random effects for MIS+rt-PA arm, and the other block – for the medical management arm and e) heteroscedastic residual errors that are different by study arm.[90] The models will be compared using likelihood ratio tests and Akaike Information Criterion (AIC). In addition, since a 'plateau' or 'floor' effect of clot resolution over time is likely, subsequent models to statistically test for these fixed effects will consider clot resolution in non-linear terms, such as linear/cubic splines and other non-linear functions. From the final model, the empirical Bayes estimates of the random slopes will be computed to be used in the next phase. The second-phase analysis will be based on multivariable logistic regression models that include the estimated clot resolution trajectories over time, the covariates listed in equation (1) above, and the dichotomized mRS at 180 days post-stroke as the outcome.

Hypothesis 2b: There is a percent EOT clot reduction and/or EOT clot volume that maximize(s) the probability of a "good" outcome. The null hypothesis is: *There is no relationship between percent EOT clot reduction and/or EOT clot volume (as dichotomized categories) and "good" functional outcome.*

Analysis 2b: In this analysis, the clot resolution will be represented as either percent clot resolved at EOT or the EOT clot size. The following cut-points will be investigated as best predictors of "good" outcome at 180 days: 60, 70 and 80% (EOT percent clot resolved), and 10, 15 and 20 mL (EOT clot volume). For each cut point, a dummy variable will be included in the multivariable logistic regression model for "good" outcome adjusted for covariates in equation (1). The models will also assess whether the best cut-points are treatment dependent by including an interaction term between category or percent EOT clot resolution (or the EOT clot volume) and the treatment group.

The models in aims 2a and 2b will be expanded to consider any mediating or modifying factors for the effect of surgical intervention on functional outcome scores. The statistical investigation of mediators that may have an effect on surgery is motivated by the desire to understand whether there is a causal chain whereby surgery affects clot resolution, which in turn affects functional outcome. Although there is no direct test for the existence of such a causal chain, the data can be examined to explore whether they are consistent with such a hypothesis. The methods proposed by Baron and Kenny[91] will be used in these analyses, where a series of three regression models will be created to test for bivariable associations between surgery on functional outcome, surgery on clot resolution, and clot resolution on functional outcome, respectively.

Analysis of Secondary Outcomes. Analysis of the secondary outcomes as described above will proceed in three phases: 1) exploratory, 2) cross-sectional evaluations (30 day mortality and safety events, 180 and 365 day post-stroke outcomes, etc.), 3) full longitudinal evaluations (30 to 365 days), and 4) cost-benefit and cost-effectiveness analyses.

Exploratory Phase. The initial phase of the statistical analysis will consist primarily of exploratory methods that include the examination of these data for indications of unusual observations, inconsistencies, or gaps in any pertinent outcomes and related measures; use of visual techniques (e.g., histograms, box-plots, etc.) to determine the distribution and spread of these data; the calculation of summary statistics (e.g. means, medians, variances, etc.) to determine the central tendencies and variability for continuous variables; and the use of contingency tables to assess frequency distributions for categorical variables. Any unusual observations or outliers will be reviewed with the DCC for possible data entry errors, and verified prior to any analyses performed on the data. Exploratory data analyses will be used to summarize these data for the overall patient population and separately by treatment arm (with appropriate blinding restrictions as required), and will primarily occur in conjunction with DSMB evaluations, that is, before the end of the trial and before unblinding of the leadership team.

Cross-Sectional Phase. The second phase begins after the unblinding, and will compare cross-sectional outcomes between the treatment arms using univariate confirmatory analyses (e.g., t-tests, analysis of variance models, logistic regression models, etc.). These group comparisons will be guided by our initial exploratory analyses. Logistic regression will be used to assess 365-day differences in mRS (0-3 vs. 4-6 and 0-2 vs. 3-6). The logistic models will account for design aspects such as potential within-site clustering that might otherwise lead to biased estimates in the standard errors associated with the regression coefficients. Additionally, these models will control for potentially confounding variables, including pre-randomization ICH volume, IVH volume (or presence, depending on the variability in IHV volumes), baseline stroke severity, age, and co-morbidity (diagnosis of Diabetes or history of cardiovascular disease).

When we consider the secondary analysis of the ordinal mRS, we will estimate a proportional odds model of the form:

$$\log\left(\frac{\gamma_k}{1-\gamma_k}\right) = \alpha_k + \beta X + \theta \cdot TRT \qquad (6)$$

where a_k = Prob(mRS < k), k=0,1,...,5.

The proportional odds model is very similar to the logistic regression model described above, with the difference being that a treatment effect leads to an increase in the likelihood of a patient being in any subsequently lower mRS category. The probabilities, a_k, are now cumulative probabilities which incorporates all outcomes below any given level. The set of intercepts, a_k k=0, 1, …, 5, define the initial probabilities of these cumulative levels, covariates are again included through β and X, and the treatment difference θ now describes the log-odds of moving from any cumulatively higher (worse) category, into any cumulatively lower (better) category. For instance, here θ describes both how the MIS+rt-PA changes the probability of ICH patients away from a potential mRS category of 'Dead' (6) to 'No Symptoms through Severe Disability' (0-5), as well as how the MIS+rt-PA changes patient probabilities away from 'Severe Disability or Dead' (5,6) to 'No Symptoms through Moderate Disability' (0-4) and so forth. This model is more efficient than the simple dichotomous approach since it uses the available information across the scale of the measure. To examine the proportional odds assumption, we will additionally examine generalized logistic models that replace β and θ with β_x and θ_x, allowing non-parallel effects between successive cumulative categories. In the event that the proportional odds assumption is rejected, we will present results from the generalized logistic models.

Survival models will be constructed to examine the mortality, for the MIS+rt-PA and medically-managed groups. Standard Cox proportional hazard models will initially be examined. These models are commonly written as:

$$h_i(t) = \lambda_0(t)\exp(\gamma X + \phi \cdot TRT) \qquad (7)$$

where $\lambda_0(t)$ is a non-parametric baseline hazard function, γ is a vector of regression coefficients related to the X covariates and ϕ is the parameter for the treatment effect of MIS+rt-PA on the hazard of death (mortality). This model may be extended by including time-dependent covariates and non-proportional hazards as deemed necessary through diagnostic checks. Observed mortality will also be compared with predicted mortality based on clinical presentation, overall and by treatment group. Predicated mortality will be estimated using the most recent Tuhrim model which takes IVH volume and presence of hydrocephalus into account.[92]

Longitudinal Phase. Finally, the third analysis phase will examine the secondary outcomes that are longitudinally collected at baseline and the follow-up time points (e.g. at 30, 90, 180, 270, and 365 days post-stroke). These data will have within-patient correlations from one time point to the next, making it necessary to use appropriate

analytical methods that properly estimate the coefficients and their associated standard errors in the analysis models. Since the overall goal of the trial is to compare subjects receiving the MIS+rt-PA intervention with subjects receiving medical standard of care, a marginal model estimated using a generalized estimating equations (GEE) technique[93] is appropriate. The GEE technique requires specification of the standard 'mean' model, as well as specification of a working 'association' model. One strength of GEE is that the parameters of main interest in the mean model (i.e. the intervention effect) are consistent regardless of whether the association model is specified correctly. Generalized Linear Mixed Models (GLMMs) may also be used to estimate subject, surgeon, and site-specific effects.[88,89]

Cost-effectiveness Analysis. Cost-benefit and incremental cost-effectiveness analyses will be performed using data on length of hospital stay, patient disposition (home, long-term ambulatory and nursing care, death), time to return home, and cost of procedures and hospital stay. These analyses will be performed with respect to both 180 and 365-day periods post-stroke. For cost-benefit analyses, the cost of ICU and the rest of the hospital stay, including the cost of MIS+rt-PA will be considered. Cost data will be obtained from the trial (secondary outcomes) and from the literature.[94,95] For the incremental cost-effectiveness analysis, the distribution of hazard rates of changes in mRS and in-hospital stay will be applied between the treatment arms, considering both short-term (acute care) and long-term care post-stroke. The model will include a one-year time horizon and a secondary analysis of life-time horizon. The analysis will be generated from the health care system perspective for costs combined with the quality-of-life measures for effectiveness (i.e. SIS, EQ-5D, and Personal Health Utility Interview). Various measures of effectiveness will be employed and the robustness of the findings to changes in the source of patient utility/quality of life measures will be reviewed. This will be supplemented by looking at ranges for patient utilities reported in the literature.[96] Finally, probabilistic sensitivity analyses will be conducted using a bootstrap approach, producing a scatter plot of incremental costs and effects to assess the impact of statistical uncertainty on the robustness of the conclusions.[96]

9.1.8. Safety Analyses. The primary safety measures for this study are symptomatic intracranial bleeding, infection, and 30-day mortality. The safety thresholds for these measures have been set as: 30-day mortality > 60%, first week operative death rate > 10%, a MIS and rt-PA related symptomatic brain bleeding rate > 25% (events identified within the first 30 days and assessed as occurring during active treatment or during the 72 hours after treatment will be monitored), and a procedure-related brain infection rate > 15% (over the initial 30 days).

9.1.8.1. Adverse Events (AE) and Serious Adverse Events (SAE). All AEs and SAEs are summarized by type and by treatment group in terms of frequency of the event, number of subjects having the event, timing relative to randomization, severity (mild, moderate, severe), and relatedness to the study treatment (definitely, probably, possibly, definitely not). At the end of the study, the cumulative incidences of these events will be compared between the two

treatment groups using Fisher's exact test. Additionally, generalized linear mixed models for binary data will be used to examine AE and SAE probabilities between treatment groups while accounting for potential confounders and sites center clustering effects.

9.1.8.2. Time to Death and Time to Re-Bleed. As a secondary assessment, time to death or re-bleed within the one-year follow up period are compared between the MIS+rt-PA and medically-managed groups adjusting for appropriate baseline covariates. Provided the model assumptions are met, a proportional hazards regression model may be used for the analysis as described above.

9.1.8.3. Safety Monitoring. The DCC will generate periodic DSMB reports (We assume that the total number of subjects enrolled per six months in a single treatment group will be approximately 30; thus, eight semi-annual looks at the data will be suggested to the DSMB. As usual though, periods for reporting will be discussed and finalized at the initial DSMB meeting). Each report will provide cumulative summary statistics on enrollment; subject status in the study (e.g., number completed at 3-month, 6-month, 9-month, and 12-month assessments); baseline characteristics; protocol violations; safety data, including AEs and SAEs by AE code, severity, and relatedness to the study medication; outcomes data (if coinciding with an interim analysis – also to be discussed and finalized at the initial DSMB meeting); and data management/quality information (e.g., timeliness and completeness of data entry by the clinical sites centers via the MISTIE III Trial Website; number of queries generated and resolved). Closed reports will be generated for the DSMB voting committee members with data provided by blinded treatment group (noted as A and B). Only the unblinded statistician has access to the efficacy endpoint data. These data will not be included in the reports for the safety reviews. The outline of these proposed DSMB reports will be included in the Manual of Operations and Procedures prepared by the Clinical Coordinating Center (CCC). In an interval as yet to be determined (monthly or quarterly), the CCC will also generate a Safety Monitoring Report to be distributed to the DSMB. This report contains only the enrollment, subject study status, safety, and data quality information. The Executive Committee also receives the Safety Monitoring Reports. The DSMB will have discretion to recommend stopping the trial early if safety concerns become substantial. Safety stopping rules for each of the primary safety outcomes will be developed and used to help the DSMB make its safety assessments. The DSMB makes recommendations to NINDS who has authority to stop the investigation for safety or any other reason after discussion with the trial PI.

9.1.9. Statistical Power for the Primary Efficacy Aim. The MISTIE Phase II results, for all available data (trial-standardized "pilot" and randomized), demonstrated a best estimate for the increase in the probability of achieving an mRS 0-3 of of 13.0% [95% CI: (0.4%, 26.4%)] comparing MIS+rt-PA treatment versus medical

management 180 days post-stroke. When only randomized patients are considered (N=90), the best estimate for the unadjusted effect size is approximately 11.0% [95% CI: (-7.9%, 29.7%)], and approximately 12.0% [95% CI: (-2.7%, 26.9%)] after adjusting for baseline clinical characteristics. These estimated effect sizes are statistically equivalent given the similarity in the CIs. Nevertheless, their range provides a strong motivation for conducting the proposed Phase III trial to confirm these clinically beneficial findings. The analogous unadjusted and adjusted effect sizes based on non-lobar ICH patients are very similar and statistically equivalent to these effect sizes.

In the proposed design, subjects will be randomized to MIS+rt-PA versus medical management using a covariate-adaptive design. The goal of this randomization scheme is to obtain an improved balance across study arms in the number of subjects with certain pre-randomization variables that are strongly predictive of the functional outcomes at 180 days post-stroke. These variables include pre-randomization clot size and baseline severity of impairment as measured by GCS. The design of Pocock and Simon[83] will be used, which increases a newly enrolled subject's probability of being assigned to the study arm that improves the overall balance in these important prognostic factors.[84] The trial will initially enroll ICH patients until either a maximum of 500 patients is accrued or the trial is stopped early for efficacy or futility using group sequential boundaries described below (Table 1).

Table 1. Efficacy and Futility Boundaries for the Study Population with a Risk-Difference Assuming the Conservative Effect Size of 11%

Boundary	After 250 patients	After 375 patients	After 500 patients
Efficacy	2.87	2.34	2.03
Futility	-2.30	-1.98	

The above stopping boundaries are used after 250, 375 and 500 patients, respectively, have 180-day post-stroke measurements. If the test statistic described above exceeds the efficacy boundary, or if it is less than the futility boundary, the enrollment of patients is stopped early. The futility boundary at each interim analysis corresponds to a one-sided test at level 0.0001 of the hypothesis that the probability of a "good" outcome (mRS 0-3) is ≥ 11% under assignment to MIS+rt-PA vs medical management.[97] These boundaries are more aggressive than the efficacy boundaries (the latter of which are based on O'Brien-Fleming boundaries, but are proportionally increased to strongly control the study-wide Type I error rate at the 5% level).

The above design has the following properties as described in Figure 1 and Table 2:

i) At least 88% power to detect a treatment benefit ≥ 13%, assuming the probability of mRS 0-3 under medical management is 25%;

ii) At least 81% power to detect a treatment benefit ≥ 11%, assuming the probability of mRS 0-3 under medical management is 20%.

Table 2. Estimated Power for Risk-Difference Effect Sizes[a] from 10-13% (N=500) by Probability of mRS 0-3 in Medical Management Arm

	Base Plan[b]	Plan B[b]
Effect Size	**Probability = 0.25**	**Probability = 0.20**
13%	**88%**	91%
12%	83%	87%
11%	76%	**81%**
10%	68%	73%

[a] MIS+rt-PA – medical management
[b] Tables 4, 5 in CCC grant

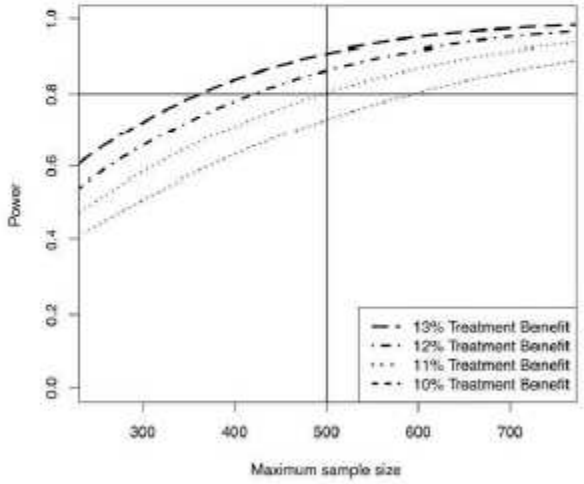


Figure 1. Power curves at ranges of effect sizes on risk difference scale, assuming baseline probability of modified Rankin Score 0-3 for medically-managed patients = 25%

9.1.10. Statistical Power for Secondary Aim 2a. Based on MISTIE II data (Table 1), the relationship between mRS score and EOT clot size is estimated as an OR (95% CI) of 3.82 (0.97-15.1, p=0.056). Simulations were performed on 1000 generated samples of 250 patients per group based on randomized MISTIE II patient data. These simulations indicate that with 500 total patients the estimated power to detect an adjusted OR of 3.82 is ≥ 98%. Additional simulations suggest that there is ≥ 90% power for detecting an adjusted OR as low as 2.38 to evaluate the strength of the relationship between clot size, dichotomized as ≤ 15 vs > 15 mL and mRS outcome (0-3 vs 4-6).

9.1.11. Innovative Methods. Categorical outcomes such as the mRS, GOS, etc, are termed composite outcomes in the statistical literature as they combine outcomes of interest such as ability/disability with mortality outcomes. There have been recent methods developed to allow simultaneous examination of the ability/disability and mortality outcomes but they do not specifically include

mortality in the actual outcome scale. Joint analyses based on generalized linear mixed models (GLMM) have been introduced,[98] and are proposed to extend these models for the brain injury/stroke literature using data from this trial. A latent Gaussian process will be posited to represented an individual's underlying propensity to regain ability which describes relationships between a subject's longitudinal outcomes. A random effects model corresponding to the dichotomous mRS logistic regression model outlined above may be specified as:

$$\left(\text{logit}\left(Prob\left[Y_{ij} \leq 3\right]\right)\right) = \beta_0 + \beta_{0i} + \theta Trt + \beta X_{ij} \qquad (8)$$
$$\beta_{0i} \sim N(0, \tau^2)$$

where Y is the binary outcome for patient *i* at the time *j* and Y_{ij}=1 if mRS_{ij}=0-2 and Y_{ij}=0 if mRS_{ij}=3-5. Note that the category mRS_{ij}=6, indicating death, has been removed from the outcome definition such that Y_{ij} describes ability for those remaining alive at time *j*.

9.1.12. Missing Data. Based on previous MISTIE and CLEAR studies performed in this group of stroke patients, the CCC will make substantial efforts to ensure complete collection of data for all patients, and to accrue minimal LTF to optimize evaluation of the primary outcome of 180-day adjudicated mRS post-stroke. In the CLEAR III trial, there is a LTF of 2% among the first 100 patients as reported to the DSMB. Rates of missing data and LTF will be reported, and no imputation of the primary outcome measure will be undertaken. However, multiple imputation methods based on regression modeling[99] will be used to estimate missing covariates incorporated in the logistic regression model for estimating the adjusted treatment effect. In addition, the effects of incompleteness/noncompliance will be quantified through sensitivity analyses, the gold standard in the field.[100]

9.1.13. Multiplicity. The study design for the primary comparison is a two-arm randomized trial with a single-treatment contrast, hence, multiplicity from several tests is not an issue.

9.2 Data Monitoring

Data safety and monitoring procedures will be in place before enrollment begins and monitoring will be performed on a regular basis throughout the subject accrual and treatment periods.

Medical Monitor: Dr. Carlos Kase, MD, a stroke physician not involved with the study, will serve as the Medical Safety Monitor. Dr. Kase is a neurologist with experience treating acute ischemic and hemorrhagic stroke as well as clinical trials. He is familiar with the proposed study intervention. He will be responsible for ongoing monitoring of reports of SAEs and MEOIs submitted by the clinical centers to ensure good clinical care and to quickly identify safety concerns. If necessary, he will suggest measures to be taken to prevent the occurrence of particular adverse events, e.g., modifying the protocol to

require frequent measurement of laboratory values predictive of the event. In the event of unexpected SAEs or an unduly high rate of SAEs, he will be responsible, with the assistance of the CCC, for notifying the DSMB Liaison.

ICU Care Medical Monitor: Dr. J. Ricardo Carhuapoma, MD will serve as the ICU Care Medical Monitor. He will review all serious adverse events, focusing on American Association of Neurology (AAN) guidelines and ICU protocols prior to, during, and following each SAE. He will also review ICU care and drug administration protocol deviations and mentor the site PIs on protocol compliance. Dr. Carhuapoma is a member of the Johns Hopkins University neurocritical care team and manages critically ill patients with neurologic and neurosurgical diseases. She has extensive published experience with the CLEAR trials and procedures, and radiologic evaluation of the MISTIE procedure. He participates in clinical and basic science research with collaborators in the departments of stroke neurology, neurosurgery, and anesthesia. His clinical interests include intracranial pressure, ICH and IVH, and ICU resource allocation. He has authored multiple MISTIE Phase II publications. He will work closely with the leadership and the Safety Compliance Officer to assess ICU protocol performance in relation to functional outcomes in MISTIE III. Dr. Carhuapoma will also be on call to assist in enrollment and protocol performance decision-making.

10 DATA COLLECTION, SITE MONITORING, AND ADVERSE EXPERIENCE REPORTING

10.1 Records to Be Kept

Records Retention. Participation in this study requires that original study documents be retained for a minimum of 2 years following notification of the FDA by the study CCC that investigations have been discontinued. This standard complies with U.S. FDA regulations (21 CFR §312.62[c]). Records must not be destroyed without first contacting the CCC to ensure that the time limits defined in the regulations have been met. Study centers in countries other than the US participating in the trial may have to comply with different requirements but should comply with whichever requirement is longer.

For the purposes of this section, “original study documents” are defined as:

- Subject medical records created at or available to the enrolling center during the subject’s participation in the trial, or any other document that supports entries in the EDC system and represents the original source of that information, including but not limited to applicable sections of medical charts, patient correspondence, laboratory data, pharmacy logs and drug accountability forms, as well as any forms or documents used to compile or maintain original subject data or study procedural information. Intermediary documents and worksheets used to organize and compile original records into a form that facilitates easier transcription into the EDC do not represent original study documents. Certain data may be entered

directly into the EDC in which case the EDC system represents the original study document.

- All Essential Regulatory Documents (as defined under Good Clinical Practice Regulations) including: all material communications with the IRB; all communications with the Sponsor (including the surgical center, reading center, outcomes committee, endpoint committee, safety monitor, Emissary's monitoring staff, etc.) that are related to study subjects or which otherwise document material study-related procedures or safety issues; and, all training records and documentation that all participating staff are suitably qualified and authorized (CVs, 1572, Delegation Log, etc.).

- Archival copies of the data and electronic documents from the VISION-EDC system.

All study documents should be uploaded to the Electronic Trial Master File (eTMF) section of the VISION-EDC system. VISION will be used as the master repository for all site and Sponsor regulatory documents, and all patient source documents with the exception of DICOMs and any records not uploaded to the EDC (perhaps for confidentiality reasons or do to specific site discretion, such as might be suitable for financial contracts), sites generally do not need to maintain duplicate local files unless otherwise mandated by local institutional requirements.

At the conclusion of the study, all entered patient data and uploaded documents (with the exception of Modified Rankin videos and DICOMs) in the VISION-EDC system will be archived and provided to the site on DVDs. Modified Rankin video interviews uploaded to the Glasgow outcomes center will be destroyed at the conclusion of the trial in accordance with informed consent commitments. Due to their extreme size, DICOMs submitted to the EDC system will not be maintained long-term in the EDC system, but rather will be promptly deleted once they have been reviewed by the reading and surgical centers. Sites will be responsible for retaining DICOMs via their local PACS system (or local copies of CDs).

Regulations require that study documents (including the archive CDs and any study documents not uploaded to the EDC) must be retained in the files of the responsible investigator for potential review by regulatory agencies. As this is an international study conducted under the jurisdiction of multiple regulatory bodies (FDA, NIH, Health Canada, ICH, etc.) and for not in support of any one specific regulatory application, retention requirements may be considerably longer than what may be required under local or regional regulations or other trials being conducted at the site. As such, the principal investigator must retain the study documents until otherwise instructed by the coordinating center. The expected retention period is a minimum of 2 years after the final report is submitted to the FDA after the conclusion of the overall clinical trial, irrespective of any particular site's participation.

10.2 Role of Data Management

10.2.1 Investigator/Institution will permit trial-related monitoring, audits, IRB/IEC review, and regulatory inspections by providing direct access to source data and documents.

Data Collection and Reporting: Data for each patient will be reported and recorded on electronic case report forms. Electronic case report forms (eCRF) must be completed for every randomized patient. This means all patients who have a signed informed consent, undergo screening procedures, and fulfill all eligibility criteria.

The electronic CRF (eCRF) will be completed by the site investigator(s) listed on the Form FDA 1572 or otherwise designated by the site Principal Investigator. If any entry on a source document requires change, a single line will be drawn throughout the incorrect entry, and the correction will be entered in ink, initialed and dated. Whiteout, erasures, or obliteration on source data are not permitted.

10.2.2 **Statistical Center responsibilities as the Data Coordination Center (DCC).** The responsibilities of the DCC in the MISTIE III trial include: trial design development and monitoring of trial performance, data management planning, review and verification of CCC data for analysis/presentation, statistical design and analysis, and reporting of results for various aspects of trial management (e.g., safety, site performance, surgical performance, fidelity of adaptive randomization) and trial evaluation (e.g., DSMB reporting, interim analyses, publications, resource sharing). The DCC will collaborate closely with CCC throughout the duration of the trial, and will rely on CCC leadership to provide the appropriate scientific and clinical guidance to ensure the successful completion of the responsibilities described above.

10.3 Quality Assurance

MISTIE III will have a robust safety and data monitoring program in full compliance with NIH policy of 10-Jun-1998 as expanded by NINDS guidelines published 14-Sep-2011 in addition to compliance with all applicable U.S. and international GCP regulations. This program, designed to safeguard the well-being of study participants and to ensure scientific integrity, will include the following components:

- A NINDS-appointed independent DSMB;
- A dedicated safety committee comprised of two independent medical monitors (a neurologist and a neurointerventionalist/neurosurgeon) with a dedicated independent safety compliance officer;
- Oversight of the CCC and the multicenter trial by an independent IRB in addition to the individual site IRBs;

- A surgical committee with a dedicated surgical center providing oversight during patient enrollment;
- An outcomes committee composed of an independent monitor to review the integrity of the multiple outcome assessments and a centralized, dedicated Glasgow outcomes center providing standardized adjudication of mRS assessments;
- An imaging reading center providing independent assessment of clot resolution, edema, and mass effect;
- Automated data quality checks at the time of EDC form completion by the investigational site;
- Dedicated independent data monitoring and QA teams (Emissary International and The George Institute);
- A robust training program prior to site activation, interactively during each medical and surgical enrollment, and with monthly advanced training topics, monthly newsletters, and quarterly performance reviews.

The components of this multifaceted approach, including each dedicated team's specific responsibilities, are discussed below.

10.3.1. Data and Safety Monitoring Board

A DSMB will provide an independent review of the research, interim safety and efficacy data, and progress towards achieving the goals of the study. To enable the CCC to properly manage the trial, the project leadership, and key personnel will jointly work on a DSMB plan early in the process. After the award but before the project begins, CCC will be coordinated with the DCC to develop a detailed monitoring plan. The NIH-appointed DSMB will then approve the plan. The monitoring plan will describe the process for reporting adverse events to the IRB, FDA, and NIH.

DSMB Administration: DSMB(s) will monitor safety and performance and review interim trial analyses. At periodic patient-completion milestones, the DSMB will meet to review the data for safety, efficacy, and quality. Investigators and staff involved in the interim analyses will attend open sessions of the DSMB meetings to present analyses and, if needed, respond to questions and directly obtain requests for new analyses or revised presentation formats. The trial leadership will follow the pretrial steps recommended by the NIH's NINDS. The CCC has extensive experience with DSMB preparation and reviews.

Interim safety analyses will be prepared for the external DSMB on a pre-arranged schedule (such as semi-annually or after enrollment of a fixed number of subjects) to evaluate efficacy and safety. Safety measures include: monitoring incidence of symptomatic and asymptomatic intracranial bleeding events (i.e., hemorrhage extension, new hemorrhage, catheter tract hemorrhage) through daily computerized tomography (CT) scans for the first four to five days after enrollment and until day seven (about 24 hours after ICH catheter removal), monitoring the incidence of confirmed and suspected

infection, and in-hospital mortality. Safety events will trigger a "suspend recruitment and review" by the DSMB. The DSMB will then investigate the presumed cause and impact of these events are: 30-day mortality > 60%, a MIS and rt-PA related symptomatic bleeding rate > 25% (events identified within the first 30 days and assessed as occurring during active treatment or during the 72 hours after treatment will be monitored), first week operative death rate > 10%, and a procedure-related infection rate > 15% (over the initial 30 days). If any of these events are attributable to the catheter insertion or manipulation, or to the rt-PA injection at a 95% confidence level, then the study will be suspended for a complete safety and efficacy review. The FDA IND division will be notified according to FDA regulations, part 312.

Prior to DSMB report generation, the CCC will work with the enrolling sites to finalize the case forms, compile the data, conduct all requested analyses, and compile suitable reports, tables and graphs. General rules for this analysis are laid out in the DCC grant. All deaths will be reviewed. The most likely complications from this therapy are increased mortality, rebleeding, and cerebritis. We propose to analyze the safety data in two groups to approximate complications attributable to surgery and to the ongoing drug irrigation. The first consists of bleeding and complications within the first 24 hours after catheter placement. The second consists of bleeding and procedure-related complications in the subsequent six days.

10.3.2. Safety Committee and Safety Officer

Safety Compliance Officer: The DSMB and IRBs (see below) require a process for timely communication of adverse events and complication rates to the local sites as well as regulatory agencies. Thus, an essential component to the process is the safety compliance officer. The safety compliance officer will have the day-to-day responsibility for ensuring that adverse events and similar safety-related information are promptly collected, medically reviewed by the medical monitor(s) and the MISTIE III safety committee, and appropriately reported to regulatory agencies in compliance with GCP standards. We have found that assigning an experienced project manager to this responsibility allows the physician-experts on the safety team to focus on medical review, while the safety compliance officer focuses on maintaining quality, efficiency, and regulatory compliance. The safety officer will be responsible for preparing and communicating incoming documents and reports to the study leadership. Inclusion/exclusion criteria, review-of-system reports, medical history, medical events, and radiographic imaging entries will be cross-checked and reviewed by applying real-time database algorithms, crosschecking sentinel dates and times, procedures, AEs, SAEs, and protocol timelines. As aggregate data reports and quarterly and annual progress reports are prepared, the safety compliance officer will query the enrolling site personnel as needed and follow the data entry tasks to completion. At the time of each review, the safety compliance officer will coordinate the review of the available information and imaging with the safety committee members and follow the voting process through to consensus. The safety compliance officer will serve as the liaison between the safety committee and the enrolling center personnel to garner additional

information as needed and to elicit agreement or disagreement of the site personnel with the committee's assessment.

Online Safety Committee Review: Our safety compliance officer will customize and implement a SAE/MEOI reporting/review algorithm, already in use for current trials that include online review and electronic signature (using the regulatory-compliant electronic signature capability within the EDC) by the medical monitors and safety event committee members. The safety compliance officer will manage the development and revision of trial protocols, MOP, SOP, and protocol working guidelines with the trial leadership.

Reporting Dictionary and Data Standards: The safety compliance officer will work closely with the trial vendors to develop the EDC systems and the corresponding paper bedside worksheets, used at the bedside for all safety-related events and processes. The Common Terminology Criteria for Adverse Events (CTCAE) dictionary has been adapted for the hemorrhage trials to allow for trial-specific reporting using standardized Medical Dictionary for Regulatory Activities (MedDRA) coding. The safety compliance officer will also produce various presentations to educate clinical site personnel on the protocol and the AE/SAE/MEOI reporting process.

10.3.3. Surgical Committee/Surgical Center

Organization: Two neurosurgical teams (Cincinnati and Chicago; neurosurgeon and radiology specialist at each site) will share the review of every potential patient just prior to randomization to assess eligibility for the trial, assess case-specific risk factors, and provide specific guidance to the site investigators regarding the overall stability of the clot and patient, surgical procedure, catheter placement, and dosing decisions.

Technical Uniformity: The surgical center will review each procedure for the two technical aspects of surgery: catheter placement and drainage. Reviews will be performed in parallel with randomization and scheduled safety reviews. The, CT scans, surgical record, surgical case report forms, and the location of the catheter will be evaluated. Adequate catheter location will be achieved when the tip is located in a region defined as the "central core," within two-thirds of the overall hematoma diameter (i.e., the catheter tip must have more than one-third of the diameter of the ICH separating it from the margin of the clot). The clot dissolution rate, drainage records, ICP records, and nursing case report forms will serve as the data supporting uniformity of drainage. The surgical center will review these data concurrently with the catheter placement data.

Technical Feasibility of Catheter Placement and ICH Drainage: The MIS catheter placement will be evaluated for technical feasibility by determining the number of catheters placed in one pass with adequate tip location, giving allowance for one-time repositioning of the catheter, followed by successful aspiration to first resistance. Clot drainage will be considered technically complete or effective when it reaches the goal of less than a 15 cc volume.

Operative Procedural QA: Quality assurance will be maintained at each treatment site by additional review of the medical record by the surgical center after subject treatment. This includes: 1) review of the operative report to confirm proper use of the image guidance system and ensure the provided introducer cannula and soft ventricular catheter were properly used in each case; 2) review of the radiological findings comparing pre-surgical hematoma volume and post-surgical-intervention hematoma volume to ensure accurate blood aspirate; and 3) review of the surgical record, the surgical case report forms, and radiographic films to ensure proper catheter placement.

10.3.4. Glasgow University Outcomes Committee/Outcomes Center

We propose, via collaboration with the stroke trials team in Glasgow, to take a robust approach to efficacy endpoint assessment through training, certification, and central adjudication.

Observer Training: The Glasgow team has published on issues, including the choice of outcome scale, the optimal cut-points on these scales and the choice of analysis approach. They have also developed a training and certification program for scoring of the mRS, which has been used in three major acute stroke trials involving several thousand investigators and over 5500 subjects (CHANT, SAINT I & 2). Their preliminary data convincingly illustrate the extent of inter-observer variation and endpoint misclassification, and suggest that training and centralized adjudication help to limit variability.

Efficacy Endpoint Verification: Our Glasgow colleagues have developed and validated digital video recording of mRS outcome assessment interviews and duplicate centralized review of the outcome score – a method which also delivers objectivity in a trial with open design. There are four potential advantages to this method, some of which would specifically enhance design of the MISTIE III trial: 1) investigators are aware that they are being monitored and will conduct their interview thoroughly, ensuring that they cover the crucial elements to justify their score; 2) the video recording permits central scoring by independent assessors who can be guaranteed to be completely blinded to treatment (objective blinded endpoint); 3) the central assessment can be scored by a committee of assessors reviewing responses in the language of the subject; and, 4) to ensure agreement over controversial cases and consistency across the trial. While the central assessment will primarily be intended to make a rigorous distinction between mRS 0-3 and mRS 4-5, it may allow more detailed assessment of disability and the validation of a more sophisticated ranking methodology, which they are developing.

Video Recording and Quality Control: The mRS score as well with other assessments will be recorded locally during the subject video-interview assessment and entered into the EDC system in standard fashion. The digital recording of the assessment will be uploaded to the Robertson Centre for Biostatistics in Glasgow via an encrypted secure web form. An endpoint assessment committee will convene to review the recording and assign a mRS score based only on the information contained within the video. The score

will be entered into the EDC system and compared to the local score. There are three possible conclusions to this process: 1) if there is agreement between the local and central mRS scores, this is accepted as the final mRS outcome; 2) if there is disagreement, the endpoint committee will reconvene and the case will be reviewed. If disagreement persists and the assessment has been technically adequate, the central score will be considered final and feedback will be provided to the local investigator; 3) if there is disagreement and the endpoint committee concludes that the recorded assessment is inadequate, a data query will be issued and the site will reassess the subject. This information will be considered and a final mRS score then assigned using the same process. The use of recording technology does introduce some practical considerations that have been addressed in the Glasgow pilot study. In the pilot, technical problems arose in only 2% of assessments; after nearly 200 patients and two years in CLEAR III, there have been no technical problems that prevented obtaining the Glasgow mRS score. As outlined, all subjects will have a score recorded locally that can be used as a backup.

The additional time and cost involved in the recordings is minimal and a fraction of that required for screening, selection, recruitment, and treatment. The need for the camera, and by implication a restricted group of trained observers, may appear a disadvantage but conversely it reinforces the need for endpoint assessments to be thorough and undertaken by properly accredited individuals. The addition of observer training will minimize inter-observer variability and the risk of endpoint misclassification and the few extra minutes and dollars involved in recording of mRS assessments will be repaid handsomely in trial power and reliability. Furthermore, if MISTIE III shows benefit, comparison of the results of the local and central assessments will provide a useful estimate of the observer bias that could be inherent in the mRS assessment. The CCC and a majority of the investigators have experience with this method as it is used the current CLEAR III trial. We will take procedural steps to ensure the confidentiality of patients undergoing these video interviews.

10.3.5. Reading Center: Centralized, Adjudicated CT Analysis

Although routine CT scan determinations will be performed locally, radiographic determinations needed for treatment comparisons will be made in a central radiological setting and be part of the permanent data files. Central reading will assure a high degree of uniformity and standardization of the measurement of the hemorrhage size and assessment of edema and mass effect. All imaging studies will be catalogued and analyzed, and the results entered into the study database. This process has been tested in our prior trials. Measurements are made by experienced radiology technicians and then subsequently verified by an independent radiologist.

10.3.6. Automated Data Quality Checks in the EDC System

VISION® EDC System: The EDC system has a robust set of data quality checks that will be executed at the time of data entry at the investigational site. This includes the standard validations available with most EDC systems such as range checks (e.g., to flag

a high blood pressure as exceeding inclusion criteria or to ensure a temperature is not an impossible value) and data format checks (to flag an invalid date). Additionally, our VISION platform will perform very sophisticated cross-form computed calculations that would not be available in lesser EDC systems. For example, VISION will conduct a series of verifications looking at lab values, CT readings, demographics, etc. and give the site guidance regarding the eligibility of the patient for randomization using complex cross-page computations. It will also detect when the site has made a protocol variance and present a list of such issues to the investigator and the monitoring team for evaluation and follow-up. Consequently, our EDC system will handle much of the consistency, completeness, and logic checks immediately at the time of data collection that normally would have to be done by the monitoring team and/or offline using statistical analysis that do not typically occur until weeks or months after the patient visit. This capability therefore will result in cleaner data that will be more likely to distinguish a treatment effect and significantly reduce the cost of, and delays for, monitoring and data cleaning activities.

10.3.7. Independent Data Monitoring and Quality Assurance Team

Emissary: It is important to use an independent Contract Research Organization (CRO) for monitoring and quality assurance, to provide the highest level of integrity, industry-proven best practices, and professionally trained monitors. Our CRO, Emissary International, maintains a team of high-caliber, fully-qualified monitors in the U.S. and geographically dispersed near most of our international research sites. The Emissary team also includes foreign-based monitors and monitors who speak multiple languages. This team will be responsible for near real-time review of the clinical data entered into our EDC system against source medical records (i.e., Source Document Verification or SDV) as well as generation and resolution of the associated data queries.

QA Monitoring Plan: In accordance with recently drafted FDA recommendation for risk-based monitoring approaches, this trial will employ centralized monitoring and a sophisticated EDC system to:

- Replace on-site monitoring for activities that can be done better using centralized reviewers;
- Verify source medical records remotely to ensure data integrity, reduce transcription errors, and identify any undocumented safety events;
- Target on-site monitoring at higher risk clinical sites (e.g., sites with high frequency of errors, excessive protocol deviations, patient drop-outs, poor data timeliness, etc.);
- Utilize EDC real-time data quality checks to assess range, consistency, and completeness of data at the time of entry; and
- Employ frequent statistical analyses of study data to identify sites that are outliers relative to others and to evaluate individual subject data for plausibility and protocol compliance.

10.3.8. Robust Training and Site Management

Startup Meetings: A series of study start-up meetings will be held. Study personnel from the CCC will meet with the other investigator-coordinator teams. Each meeting is expected to last one and one-half days. An investigator/coordinator operations manual will be developed for these meetings. Overhead images and slides will be presented during the start-up meetings. The visual aids provided to each site include slides on background and significance, GCP, investigator responsibilities, FDA requirements, surgical protocols, case report forms, and other specifics of the protocol. This investigator meeting/site initiation process will acquaint the site teams with the design and methods of the trial, the study organization, treatment monitoring, and integrity of data collection. Prior to each investigator-coordinator meeting, formal mRS, NIHSS, and human subjects training certifications will be required of each site investigator and coordinator. A specialized training on the EDC system, to include execution of data security and privacy protection agreements will be required before anyone may access the EDC system.

Quarterly Site Performance Assessments: Quarterly, the data from the case report forms will be organized into a site performance report that assesses enrollment, data timeliness, protocol compliance, patient management, and data quality measures. The site managers will review these reports with the study coordinator and investigator as part of our overall quality program. The site managers will work with the site to develop plans for resolving any identified performance issues, which might include additional training or site visits, and implementation of a formal Corrective and Preventive Action (CAPA) plan.

10.3.9. Data Flow for the Data Safety and Monitoring Board

Each step in the flow of data for this study is discussed below in regards to its importance in ensuring data integrity and patient safety. The steps are numbered for reference purposes, albeit some steps may occur simultaneously and data for a single patient may be in differing stages in this process.

1. Regulatory Specialist Verification of Study Documentation: Before the site can begin enrolling patients, our Regulatory Document Specialist (RDS) will verify that all mandatory startup tasks have been completed and appropriate documentation has been uploaded to the EDC system's Electronic Trial Master File (eTMF). The RDS then will set a parameter in the EDC system that will allow the site to randomize a patient and grant user access rights to the online case form. This step will ensure that no site may enroll patients until all regulatory documentation (i.e., IRB approval, investigator qualification), staff training (i.e., Emissary College training certificates, Rankin certification, Pharmacist, and Surgical Center Training, etc.) and contractual requirements are fulfilled. During the trial, the RDS will work with the site coordinator to maintain the study documentation in the eTMF repository, and can stop a site's ability to randomize should there be safety or performance concerns. As all study documentation is online, there will be no need for bulky regulatory document binders or Sponsor-vs.-site

document file verifications. At the end of the study, the eTMF content will be provided to the site on compact disks, consisting of all the collected patient data and trial documentation, for long term regulatory retention.

2. Data Entry & Source Document Upload: Once a potential patient is identified, the coordinator will register this new patient in the EDC system, triggering an automated alert to the CCC, reading center, surgical center and monitoring team. Should the patient subsequently fail to qualify for the study, the basic demographic information and reason for screen failure will be used to assess potential selection bias at the site, and for performance tracking and epidemiologic purposes.

Next, the coordinator will upload copies of applicable medical records to the eTMF to include the EDC and ambulance records, ICU records, progress notes, medication records, radiology, and other procedure reports, surgical reports, dosing records, admit and discharge summaries, and adverse event information.

Also, each site will collect CT data files (as zipped DICOMs) and upload these to the EDC system as well. These will be reviewed by the reading center and surgical centers to assess hemorrhage stability, adequacy of dosing catheter placement, clot resolution for dosing decision support, and particular patient risk factors (i.e., aneurysm, Moyamoya, etc.). The centralized, standardized, adjudicated reading center measurements will be used in efficacy determinations, trend analyses for safety reviews, and verification of patient eligibility.

Sites will be expected to enter critical screening data (and upload diagnostic and stability CTs) immediately, and enter post-randomization data within 24 hours. The EDC system will be programmed to prevent randomization of the patient if certain data has not been entered (such as critical safety/efficacy data points from the CTs) and to send automated reminders to the investigator and site managers if sites fail to enter any other data in a timely manner.

3. Correction of Automated Errors and Warnings: As data are entered, the EDC system will immediately generate automated warnings (yellow highlights) and errors (red highlights). Warnings will represent data that is outside expected limits, such as an ICH size that exceeds the inclusion criteria, or where required data are missing. Errors will indicate conditions that are intolerable (such as an impossibly high body temperature) or that are unrealistic (such as an invalid date format).

In keeping with FDA requirements for electronic systems, the EDC system will not force the investigator/coordinator to immediately change the entered data (as that could be misconstrued as encouraging data falsification) but instead simply provide feedback via on-screen messages and red/yellow field highlighting. Warnings may be unavoidable, due to patient-specific issues, but are documented nonetheless so that they can be discussed with the monitoring team. Conversely, red errors must be resolved

before the case form page can be advanced in the workflow (i.e., signed by the coordinator) so the data will be "clean" before it is exported for analysis.

Additionally, the EDC system will generate external email notifications in response to specific entries; such as to immediately alert the CCC of a possible dosing error or to notify the safety officer of a new adverse event. The EDC system also produces various instantaneous reports that are useful for data quality and safety monitoring purposes both by the site staff and the project teams.

4. Site Manager and Study Chairman Verification of Patient Eligibility: Sites will be advised to contact the CCC (which also will be notified by the EDC of the potential patient) to verify that the patient meets the eligibility requirements and to assess safety measures (e.g., INR, PTT, platelets, exclusionary concomitant medications). Site managers will go through an enrollment checklist with the coordinator and investigator, and consult with the surgical center, study chairman, or safety officer as may be appropriate.

5. Surgical Center Determination of Catheter Placement and Patient Risk: Simultaneously, during the CCC's review, the surgical center will assess catheter placement per the postoperative CTs (using the uploaded DICOM images with findings entered directly into the eCRF). This assessment will be made to ensure the catheter is best positioned to give optimum drug delivery for rapid/maximum clot resolution and to minimize the risk of bleeding complications.

6. Reading Center Verification of Hemorrhage Stability: The CT Reading Center at JHU will review the uploaded DICOM images to assess patient stability and to make the central, standardized volumetric measures used in efficacy analysis.

7. Monitoring Team Source Document Verification and Data Integrity Review: Emissary's and The George Institute's teams of monitors will review the online case forms for completeness, logic, and consistency, then verify the entered data against the uploaded source medical records and data collection worksheets. Routine queries identified in this process will be entered into the EDC system (triggering an automated notice to the site). The monitors, working in conjunction with the site managers in the CCC, will then work with the coordinators to obtain correction of all data errors and resolution of the corresponding queries.

Reviews will include data from the entire course of each patient's participation in the study. (As noted above, screen failure data will be collected but not actively monitored in this fashion because, due to HIPAA restrictions, there is no patient-identifiable information (source documents) against which it could be verified.) In accordance with a formal monitoring plan, this activity will include a review of all uploaded source documentation and will entail a 100% source verification of the primary safety and efficacy measures. Random sampling will be used to select secondary data for similar 100% source verification. Should the data accuracy for a patient/site exceed

certain minimum expectations in this step, or if any material data integrity or regulatory compliance issues are identified, additional data from a patient/site will undergo intensive monitoring and the site referred to the CCC for remediation and potential dismissal from the trial.

10.3.10. Other Components of Data Management Plan

Randomization and Data Collection: Each subject is assigned a unique study number by the EDC, which provides a centralized, web-based randomization system. Treatment allocations will be stratified and centralized across all study centers. Daily data collection of ICP management, ICU care, catheter monitoring, and neuroimaging will assess the subject's clinical response to treatment as part of the clinical trial. This data will be used to assess compliance with the study protocol, intervention stopping rules, and care directed at independence. Daily results of routine hematology, chemistry, and coagulation studies also will be collected if reported.

Protocol Compliance: Procedures will be implemented to maximize adherence to the protocol (meetings, communications via website and teleconferences, individual and group training, MOP, and SOP documents). Comprehensive training (approximately 20 hours each for both the coordinator and investigator, one to 10 hours each for other site staff) is required before a site is activated. Early review of data is made possible by real-time entry of data into a database with validations and daily monitoring. This is particularly important with respect to the mRS, our primary endpoint, as rapid central review is anticipated to allow for repeat interview of the subject should an incomplete exam be documented. Site investigators are required to report a protocol deviation within 24 hours of occurrence or as soon as it is discovered. If the QA monitor discovers an undocumented major deviation during a monitoring activity, the monitor will notify the CCC immediately. Each site coordinator will report deviations as they are discovered to the local IRB in accordance with local requirements. While there may be rational clinical reasons for an occasional deviation, a site with serious, continual problems is at risk for losing its funding. Routine reporting of protocol deviations will be made to the NINDS, DSMB, and other regulatory agencies as required by GCP.

FDA Guidance for Electronic Data Entry Compliance: The design and development of the electronic database system will reflect the FDA Guidance for Industry for Computerized Systems Used in Clinical Trials (April 1999) as well as the Electronic Records/Electronic Signatures rule (21 CFR part 11). The system is currently in use with the CLEAR III trial and the earlier MISTIE II study. A secure, computer generated, time-stamped electronic record will allow reconstruction of the course of events relating to the creation, modification, and deletion of an electronic record. Source documents will be retained to enable a reconstruction and evaluation of the trial. The system will ensure that all applicable regulatory requirements for record keeping and record retention in clinical trials are met with the same degree of confidence as are provided with paper systems. Clinical investigators will retain the original copy of all source documents uploaded onto the eCRF. Query resolution correspondence will be maintained and eCRF edits will be

tracked by the system. Changes to a required record will not obscure the original information. The record will clearly indicate the time a change was made and clearly provide a means to locate and read the prior information through the audit trail. This audit trail will be in compliance with the 21 CFR 11.10(e). The record, along with supporting documentation, will also indicate who made the changes, when changes were made. Security measures will be in place to prevent unauthorized access to the system and data. To ensure that individuals have the authority to proceed with data entry, the system will be designed to verify the electronic signature (user ID and password) at the start of a user session. The data entry system will ensure attributability. Each entry to an electronic record, including any change, will be made under the electronic signature of the individual making that entry. A separate electronic signature will not be required for each entry or change; a single electronic signature will cover multiple entries or changes. Individuals who maintain the electronic record systems as well as the audit trail will carry the responsibilities to protect authenticity, integrity, and confidentiality of electronic records. Audit trails will be available for FDA inspectors at the study site or any other location where associated electronic study records are maintained. The system will be designed to contain the prompts, lookup values, cross-field validations, flags, and on-line help to encourage consistent use of clinical terminology and to alert the user in case that data entered are out of acceptable range. External safeguards will be in place to ensure that access to the computerized system and to the data is restricted to authorized personnel. Servers will be stored in a physically secured, guarded data center.

Security Measures: Users at the participating centers will be aware of system security measures and the importance of limiting access to authorized personnel. SOPs will be in place for handling and accessing the system to prevent unauthorized access. Access to the data at a clinical site will be restricted and monitored by the system through required log-on, security verification procedures, and audit trail. The data cannot be altered, browsed, queried, or reported via external software applications without entering through the protective software, although computers at each site may also be used for the purposes other than the clinical study. Because the system will be largely through remote access, all data and applications used for the study will be logically and physically isolated in the servers in order to preclude unintended interaction with non-study use software. These servers will be strictly monitored and maintained by designated administrators at an independent third party (e.g., only Prelude Dynamics, the contracted EDC vender, has password access to the database and only its contracted commercial data center, On-Ramp Systems both of Austin, Texas, has access to the physical hardware); neither remote sites nor any member of the project team will have the ability to change such logical security of the system. Written procedures describing contingency plans for continuing the study by alternate means in the event of hardware or facilities failures with alternate hardware or at an alternate site will be provided to each site. It should be noted that the data management procedures will reflect the advanced use of computer and software technology; include database technology, and electronic file management principles; and therefore be of the highest possible standards achievable for data security and information integrity. Specifically, the data center is SAS-70 Type II compliant, HIPAA-audited and certified for maintenance of banking, credit card, and PHI).

Backup Recovery: Records will be backed up daily to prevent a catastrophic loss compromising the quality and integrity of the data. Data will be backed up onto digital media which will be stored at an offsite location. Backup and recovery logs will be maintained to facilitate an assessment of the nature and scope of data loss in the event of a system failure. Special backup plans for video files at the Glasgow data center will include secure offsite tape backup; no clinical data other than the Rankin video interview and associated tracking (blinded patient number, visit date, investigator's score, site, and interviewer name) is stored on the Glasgow system and the Outcomes Center has no access to the EDC database.

Limited Access: Each user will be assigned an individual account with a unique username and password. Any user will be locked out after 10 consecutive attempts, with any unauthorized access attempt recorded in a log file. Users will be required to exit the system upon leaving a workstation. The computer will automatically log off the current session when an idle period reaches 30 minutes. For short periods of inactivity, the automatic screensaver will be password protected to prevent unauthorized access to the system.

Audit Trails: All changes made to data in the electronic record are tracked and recorded in the audit trail. This audit trail will capture the date/time, the contents of the changes made, and the login id used to make the change. The audit trails will be created incrementally in chronological order, with prevention of overwrite, as such data overwriting is in violation of §11.10(e). Audit trail information will be reviewed by pre-authorized personnel if the need arises to verify the quality and integrity of the data.

Date and Time Stamps: All data will be saved on a central server carrying a time stamp, which will be documented in the audit trail. The EDC software will display the participating site's time zone but record transactions in exact U.S. Central Time as the server is located in that time zone. The date and time on the server will be synchronized to the time provided by the U.S. National Institute of Standards. Individual users will be unable to change the time on the server. Patient procedure times will be collected in local time and recorded in the patient medical record, such as the date/time of a CT scan, lab draw or dose. The site's clocks are not synchronized to any particular standard or even synchronized within the site, but such exactness is immaterial for this study, consistent with the GCP norms for all multicenter clinical trials and otherwise beyond the reasonable control of the CCC.

10.4 Adverse Experience Reporting

10.4.1. Assessment of Safety

Specification of Safety Variables

Safety assessments will consist of monitoring and reporting adverse events (AEs) and serious adverse events (SAEs), all events of death, and any study specific issue of concern.

Adverse Events
AE definition: An adverse event is any untoward medical occurrence in a patient entered into the study that does not necessarily have a causal relationship with this treatment. An adverse event can be any unfavorable and unintended sign (including an abnormal lab finding), symptom, or disease temporally associated with the use of the product or treatment, whether or not related to the product or treatment. The investigator must follow adverse events to resolution whenever possible.

AEs include the following:

- AEs not previously observed in the subject that emerge during the protocol-specified AE reporting period, including signs or symptoms associated with intracerebral hemorrhage that were not present prior to the AE reporting period.
- Complications that occur as a result of protocol-mandated interventions (e.g., invasive procedures such as catheter placement).Preexisting medical conditions (other than the condition being studied) judged by the investigator to have worsened in severity or frequency or changed in character during the protocol-specified AE reporting period.

Serious Adverse Events

SAE definition: A serious adverse event is an adverse event that results in any of the outcomes listed below. For purposes of this study, Regulatory Agency reporting responsibilities have been designated to the Coordinating Center.

1. Results in death (i.e., the AE actually causes or leads to death). Study Chair agrees to adhere to FDA-defined guidelines and submit an *expedited* report of any death that is *related* (even remotely) to study drug or the MIS procedure and *unexpected* if the death occurs within 30 days from the date of the original ICH event.

2. Life threatening (i.e., the AE, in the view of the investigator, places the subject at immediate risk of death. It does not include an AE that, had it occurred in a more severe form, might have caused death.).

3. Requires inpatient hospitalization or prolongs inpatient hospitalization.

4. Persistent or significant disability or incapacity: a substantial disruption of a person's ability to conduct normal life functions

5. A congenital anomaly or birth defect in a neonate/infant born to a mother exposed to the rt-PA.

6. Considered a significant medical event by the investigator based on medical judgment (e.g., may jeopardize the subject or may require medical/surgical intervention to prevent one of the outcomes listed above).

Medical events of interest (MEOI) definition: MEOIs must be reported to the CCC for Medical Monitor and Safety Event Committee review. The AE dictionary, which drives the drop down lists on the AE screen in the EDC system, pre-specifies which events are defined as MEOIs. A MEOI may or may not satisfy the definition of a SAE but will be reported using the same SAE reporting screen in the EDC system and will undergo the same review as a SAE. In summary, the MEOIs for the trial are as follows:

1. Ventriculitis/Cerebritis/Meningitis
2. Cerebral bleeding events (asymptomatic and symptomatic)
3. Any AE or SAE deemed by the site PI or Medical Monitor as possibly, probably, or definitely related to the MIS procedure or rt-PA administration(s)
4. Any AE or SAE requiring discontinuation of dosing or withdrawal from follow-up

Adverse event intensity grading: A coding dictionary, based on the CTCAE dictionary, is developed and will be used at the site-level to code in the EDC system adverse events as they occur. The dictionary assigns an intensity code to each event term. The intensity codes are event term-specific and usually range from 1-5, with 1 being mild and 5 being death. Certain AEs in the coding guide are defined as potentially serious SAEs if there is a medically qualifying factor. All AEs coded as a grade 4 or 5 will automatically require SAE reporting. These events will be reviewed by the Medical Monitor and, as needed, the Safety Event Committee.

10.4.2. Assessment of Adverse Events

All AEs and SAEs whether volunteered by the subject, discovered by study personnel during questioning, or detected through physical examination, laboratory test, or other means will be reported appropriately. Each reported AE or SAE will be described by its duration (i.e., start and end dates), regulatory seriousness criteria if applicable, suspected relationship to the MIS procedure and the rt-PA administration(s) (see following guidance), and actions taken.

Attributability of AEs to the MIS procedure/rt-PA administrations: Adverse events occurring more than 7 days after the MIS procedure are not expected to be considered related to the procedure. Also, due to the relatively short half-life of rt-PA, adverse events occurring more than 72 hours after completion of the last rt-PA administration are not expected to be considered related to the rt-PA administration(s). The investigator and/or Medical Monitor will define whether the event is best described as UNRELATED, POSSIBLY related, PROBABLY related, or DEFINITELY related to the MIS procedure

and/or the rt-PA administration(s) according to the following definitions. To ensure consistency of AE and SAE causality assessments, investigators should apply the following general guideline:

Yes
There is a plausible temporal relationship between the onset of the AE and the administration of the rt-PA, and the AE cannot be readily explained by the subject's clinical state, intercurrent illness, or concomitant therapies; and/or the AE follows a known pattern of response to the rt-PA; and/or the AE abates or resolves upon discontinuation of the rt-PA.

- Possibly Related: The adverse event has a temporal relationship to the MIS procedure or the rt-PA administration(s). However, an alternative etiology may be responsible for the adverse event. Information on drug withdrawal may be lacking or unclear.

- Probably Related: The adverse event has a temporal relationship to the MIS procedure or the rt-PA administration(s). The event is unlikely to be related to an alternative etiology. There is a reasonable response on withdrawal (dechallenge). Rechallenge information is not required.

- Definitely Related: The adverse event has a temporal relationship to the MIS procedure or rt-PA administration(s) and resolves when rt-PA administration is discontinued. An alternative etiology is not apparent.

No

Evidence exists that the AE has an etiology other than the rt-PA (e.g., preexisting medical condition, underlying disease, intercurrent illness, or concomitant medication); and/or the AE has no plausible temporal relationship to rt-PA administration (e.g., cancer diagnosed 2 days after first dose of study drug).

- Unrelated: There is evidence that the adverse event definitely has an etiology other than that assigned to either the MIS procedure or rt-PA administration(s).

Unexpected AE definition: Unexpected events are any adverse events in which the specificity or severity is not consistent with the natural history of ICH without the test intervention including catheter placement and rt-PA administration. Unexpected will be defined as the specificity or severity of an event that is not consistent with the risk information described in this protocol.

Expected AE definition. The Medical Events listed in Appendix 5 are published by the American Stroke Association and the European Stroke Initiative as natural history events of ICH/IVH or are found in the Investigator's Brochure or Alteplase Package Insert or Product Monograph for the use of rt-PA. These medical events are therefore expected in

the disease process or with use of rt-PA. Please enter these expected events on the Adverse Event formif they occur. Reports of these events will be analyzed and submitted as grouped data by the trial's statisticians.

10.4.3. Adverse Event Reporting Period

Adverse events must be recorded in the medical chart and in the EDC system. All adverse events, serious or otherwise occurring after presentation to the emergency department but prior to randomization will be documented on the Medical History form in the EDC system. All adverse events and serious adverse events that occur during the acute treatment phase (ending at day 6) will be recorded on the Adverse Event form along with all neurological AEs and SAEs that occur through the day 365 follow-up visit.

Documentation: Documentation must include the event duration (start/stop) and the intensity of each event using the grading definitions and event terms available in the AE dictionary in the EDC system. The grades range in intensity from 1 (mild) to grade 5 (death) and are specific to each event. Specific events will not have all five grades available. All AEs coded as a grade 4 or 5 will automatically require SAE reporting.

If a subject is discontinued early from rt-PA administrations for any reason, study site personnel must clearly report and document the circumstances and data leading to any discontinuation using the electronic case report forms. It must be determined if the reason for stopping rt-PA administration is an adverse event, for example, sustained ICP above 30 mmHg during injection.

Follow up of ongoing AEs: For any untoward event(s) the subject should be followed until the event resolves or is explained with the frequency of follow up designated by the investigator.

10.4.4. Eliciting AEs during follow up visits

At each follow up visit, the subject will be asked about the occurrence of AEs since the last contact. AEs that were ongoing at the last contact will be updated with a stop date or confirmed as ongoing. This will continue until the final follow up visit at day 365 or until the subject's death, whichever occurs first.

A consistent methodology for eliciting AEs at all subject evaluation timepoints should be adopted. Examples of non-directive questions include:

- How have you felt since your last clinical visit?
- Have you had any new or changed health problems since you were last here?
- Have you (or the patient) had any serious bleeding? Examples of this include blood transfusions, a sudden drop in blood pressure, blood in urine or stool, coughing or vomiting blood or any other internal or external bleeding.

- Have you (or the patient) suffered bleeding on the brain, a stroke, or any other change in function of the brain or nerves?
- Have you (or the patient) had any symptoms such as sudden onset of shortness of breath, coughing up blood, purple discoloration of the feet, loss of pulse in legs or feet or other problems with blood clots?
- Have you had any unusual or unexpected worsening your underlying medical condition?

10.4.5. Specific Instructions for Recording Adverse Events

Investigators should use correct medical terminology/concepts when reporting AEs or SAEs. Avoid colloquialisms and abbreviations.

a. Diagnosis vs. Signs and Symptoms

If known at the time of reporting, a diagnosis should be reported rather than individual signs and symptoms (e.g., record only liver failure or hepatitis rather than jaundice, asterixis, and elevated transaminases). However, if a constellation of signs and/or symptoms cannot be medically characterized as a single diagnosis or syndrome at the time of reporting, it is ok to report the information that is currently available. If a diagnosis is subsequently established, it should be reported as follow-up information.

b. Deaths

All deaths that occur during the protocol-specified AE reporting period, regardless of attribution, will be reported to the CCC. When reporting a death, the event or condition that caused or contributed to the fatal outcome should be reported as the single medical concept. If the cause of death is unknown and cannot be ascertained at the time of reporting, report “Unexplained Death.”

c. Preexisting Medical Conditions

A preexisting medical condition is one that is present at the start of the study. Such conditions should be reported as medical and surgical history. A preexisting medical condition should be re-assessed throughout the trial and reported as an AE or SAE only if the frequency, severity, or character of the condition worsens during the study. When reporting such events, it is important to convey the concept that the preexisting condition has changed by including applicable descriptors (e.g., “more frequent headaches”).

d. Hospitalizations for Medical or Surgical Procedures

Any AE that results in hospitalizations or prolonged hospitalization should be documented and reported as an SAE. If a subject is hospitalized to undergo a medical or surgical procedure as a result of an AE, the event responsible for the procedure, not the procedure itself, should be reported as the SAE. For example, if a subject is hospitalized

to undergo coronary bypass surgery, record the heart condition that necessitated the bypass as the SAE.

Hospitalizations for the following reasons do not require reporting:

- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for preexisting conditions
- Hospitalization or prolonged hospitalization required to allow efficacy measurement for the study
- Hospitalization or prolonged hospitalization for scheduled therapy of the target disease of the study

e. Pregnancy

If a female subject becomes pregnant while receiving rt-PA or within 30 days after the last dose of study drug, a report should be completed and expeditiously submitted to the Genentech, Inc. Follow-up to obtain the outcome of the pregnancy should also occur. Abortion, whether accidental, therapeutic, or spontaneous, should always be classified as serious, and expeditiously reported as an SAE. Similarly, any congenital anomaly/birth defect in a child born to a female subject exposed to the rt-PA should be reported as an SAE.

f. Post-Study Adverse Events

The investigator should expeditiously report any SAE occurring after a subject has completed or discontinued study participation if attributed to prior rt-PA exposure. If the investigator should become aware of the development of cancer or a congenital anomaly in a subsequently conceived offspring of a female subject who participated in the study, this should be reported as an SAE.

g. Reconciliation

The Sponsor agrees to conduct reconciliation for the product. Genentech and the Sponsor will agree to the reconciliation periodicity and format, but agree at minimum to exchange monthly line listings of cases received by the other party. If discrepancies are identified, the Sponsor and Genentech will cooperate in resolving the discrepancies. The responsible individuals for each party shall handle the matter on a case-by-case basis until satisfactory resolution.

h. SAE Reporting

General reporting of SAEs: Any alarming, serious, or unexpected adverse event, including death due to any cause, which occurs during this study, inclusive of the follow up period (day 365), and whether or not thought to be related to the MIS procedure or the rt-PA administration(s), must be reported immediately (within 24 hours of learning of the event) to the CCC and to the local IRB as required. Completion of a SAE form in the

EDC system for each SAE or MEOI that occurs is required to formerly report the event to the CCC. Once the SAE is reported in the EDC system an email notification is sent to the CCC staff, safety officer, and QA monitors. The CCC will then appropriately notify the Study Chairman, Genentech, Inc. (subjects receiving rt-PA from Genentech supply only), the Medical Monitor, the Surgical Center, Health Canada, the UK and European QA Monitors, The George Institute, and the FDA. The UK and European QA Monitors will notify the MHRA and Competent Authorities in all other Member States concerned as well as to the Ethics Committees concerned as necessary. The table below provides the contact information for each of these individuals.

Name	Title	Phone Number	Fax Number	Email Address
24-hour study phone	Coordinating Center	410-736-1368	410-502-7869	
Daniel F. Hanley, MD	Principal Investigator	410-614-6996 Cell: 410-615-3749	410-502-7869	dhanley@jhmi.edu
Genentech Drug Safety	Genentech, Inc.	800-835-2555	650-225-4682 or 650-225-5288	
Pat Reilly, RN, MSN	Sr. Medical Science Liaison, Vascular Medicine (Genentech)	717-566-7993	717-566-7994	part@gene.com
FDA fax number for IND Safety Reports	FDA		1 (800) FDA-0178	
Carlos S. Kase, MD	Medical Safety Monitor	617-638-5102	617-638-7758	cskase@bu.edu
J. Ricardo Carhuapoma, MD	ICU Care Medical Monitor	(410) 955-7481	(410) 614-7903	jcarhua1@jhmi.edu
Marc Lemieux	Health Canada	514-398-2667	514-398-8576	marc.lemieux@mcgill.ca
Barbara Gregson, PhD	QA Monitor, UK; MHRA	+44 191 233 6161 ext. 22175	+44 191 256 3268	barbara.gregson@ncl.ac.uk
Alan Cohen	QA Monitor, Europe	+32 4 738 650 91		alanscohen@skynet.be
Michelle Leroux	The George Institute	+61 2 9993 4509	+61 2 9993 4502	mleroux@georgeinstitute.org.au
Vandna Kishore	FDA/CBER (IND File)	301-796-4193	301-796-9842	Vandna.Kishore@fda.hhs.gov

An expedited IND safety report will be used to notify the FDA IND division of each serious unexpected suspected adverse reactions according to FDA regulations, part 312 and Guidance for Industry and Investigators: Safety Reporting Requirements for INDS and BA/BE Studies effective March 28, 2011. In accordance with these regulations, this protocol has a pre-specified monitoring plan for determining if subjects receiving the intervention are at higher risk for mortality and will only report a death as an expedited IND safety report if there is evidence of a causal relationship between the intervention and/or the study drug and the event resulting in death. In addition, an expedited IND safety report will be used to notify the FDA if there is an imbalance between the arms suggesting there is a reasonable possibility that the intervention or the study drug caused any of the safety endpoints: symptomatic bleeding, cerebral infection, mortality occurring with seven days of the surgical intervention, 30-day mortality. Otherwise, the occurrence of these safety endpoints will be reported on an annual basis.

The CCC will report all AEs and SAEs to the Study Chairman as the IND Sponsor (in accordance with CFR 312.32: IND Safety Reports) and the DSMB either immediately or as a routine summary report depending upon the severity of the event.

Any study report submitted to the FDA by the Sponsor-Investigator will be copied to Genentech. This includes all IND annual reports and the Clinical Study Report (final study report).

The CCC will submit events meeting the following criteria to the Food and Drug Administration (FDA) as expedited IND Safety Reports, Genentech Drug Safety using the fax cover sheet in Appendix 6 (if the event occurred in a subject receiving rt-PA from the Genentech supply), to the UK and European QA Monitors, and The George Institute according to the guidance and timelines below. The completed Medwatch/case report will be faxed immediately upon completion to the FDA and Genentech Drug Safety. The FDA fax number for IND Safety Reports is 1 (800) FDA 0178. All written IND Safety Reports submitted to the FDA by the Investigator will also be faxed to Genentech Drug Safety at (650) 225-4682 or (650) 225-5288. For questions related to safety reporting, please contact the CCC or Genentech Drug Safety by telephone at (888) 835-2555 or by Fax at (650) 225-4682 or (650) 225-5288.

Occasionally Genentech may contact the reporter of the SAE for additional information, clarification, or current status of the patient for whom an adverse event was reported. For questions regarding SAE reporting, you may contact the CCC or the Genentech Drug Safety representative noted above or the Medical Science Liaison assigned to the study (see table above). Relevant follow-up information should be submitted to the CCC for distribution to the FDA, Health Canada, Genentech Drug Safety, the UK and European QA Monitors, The George Institute and all participating investigators as soon as it becomes available and/or upon request.

7 Calendar Day Telephone or Fax Report: The CCC will notify the FDA, Health Canada, Genentech (if the subject received rt-PA from the Genentech supply), the UK and European QA Monitors, The George Institute, and all participating investigators for local IRB/Ethics Committee review of any **fatal or life-threatening** adverse event that is **unexpected** (as defined above) and assessed by the investigator to be **possibly, probably, or definitely related** to the MIS procedure or the rt-PA administration(s). Such reports will be emailed or faxed within 7 calendar days of the CCC first learning of the event. The UK and European QA Monitors and The George Institute will submit the reports to the MHRA and Competent Authorities in all other Member States concerned as well as to the Ethics Committees concerned as necessary. Relevant follow-up information will be submitted to all parties as soon as it becomes available.

15 Calendar Day Written Report: The CCC will notify the FDA, Health Canada, Genentech (if the event occurred in a subject receiving rt-PA from the Genentech supply), the UK and European QA Monitors, The George Institute,

and all participating investigators for local IRB/Ethics Committee review, in a written IND Safety Report, of any **serious, unexpected** AE (as defined above) that is considered **possibly, probably, or definitely related** to the MIS procedure or the rt-PA administration(s). Such reports will be emailed or faxed within 15 calendar days of the CCC first learning of the event. The UK and European QA Monitors and The George Institute will submit the reports to the MHRA and Competent Authorities in all other Member States concerned as well as to the Ethics Committees concerned as necessary. Relevant follow-up information will be submitted to all parties as soon as it becomes available.

15 Calendar Day Written Report: The CCC will notify Genentech Drug Safety within fifteen (15) calendar days of the Awareness Date all SAE reports that are related to the rt-PA and AEs of Special Interest (regardless of causality) if the event occurred in a subject receiving rt-PA from the Genentech supply.

30 Calendar Day Written Report: The CCC will notify Genentech within thirty (30) calendar days of the Awareness Date all SAE reports that are unrelated to the rt-PA administration(s) and any reports of pregnancy following the start of administration of rt-PA if the event occurred in a subject receiving rt-PA from the Genentech supply.

Quarterly Written Report: The CCC will notify Genentech of all non-serious AEs originating from the study experienced by subjects receiving rt-PA from the Genentech supply.

Annual Written Report: The CCC will notify the FDA, Genentech (events occurring in a subjects receiving rt-PA from the Genentech supply only), The George Institute, and the European Member States in whose territory the clinical trial is being conducted and the Ethics Committees concerned as necessary with a listing of all suspected serious adverse reactions which have occurred over this period and a report of the subjects' safety. Each Member State shall see to it that all suspected unexpected serious adverse reactions to an investigational medicinal product which are brought to its attention are immediately entered in a European database to which, in accordance with Article 11(1), only the Competent Authorities of the Member States, the Agency and the Commission shall have access. The Agency shall make the information notified by the Sponsor available to the Competent Authorities of the Member States.

• Written IND Safety reports will include an **Analysis of Similar Events** in accordance with regulation 21 CFR § 312.32. All safety reports previously filed by the investigator with the IND concerning similar events will be analyzed and the significance of the new report in light of the previous, similar reports commented on.

• Written IND safety reports with Analysis of Similar Events will be submitted to

the FDA, Health Canada, the MRC, Genentech (if the event occurred in a subject receiving rt-PA from the Genentech supply), The George Institute, and all participating investigators for local IRB/Ethics Committee review within 15 calendar days of the CCC first learning of the event. The UK and European QA Monitors and The George Institute will submit these reports to the MHRA and Competent Authorities in all other Member States concerned as well as to the Ethics Committees concerned as necessary.

For questions related to safety reporting, please contact the CCC.

11 HUMAN SUBJECTS

11.1 Institutional Review Board (IRB) Review and Informed Consent

This protocol and the informed consent document (Appendix 1) and any subsequent modifications will be reviewed and approved by the IRB or ethics committee responsible for oversight of the study. A signed consent form will be obtained from the subject. For subjects who cannot consent for themselves, a parent, legal guardian, or person with power of attorney, must sign the consent form; additionally, the subject's assent must also be obtained if he or she is able to understand the nature, significance, and risks associated with the study. The consent form will describe the purpose of the study, the procedures to be followed, and the risks and benefits of participation. A copy of the consent form will be given to the subject, parent, or legal guardian, and this fact will be documented in the subject's record.

The site investigator will provide the CCC with documentation of institutional review board approval of the protocol and the informed consent document before the study may begin at the site. The ethical review board(s) will review the protocol as required.

The Investigator is to supply the following to the study site's institutional review board(s):

1. The current clinical investigator brochure or Product Monograph
2. The current protocol and informed consent document
3. All updates to the clinical investigator brochure or Product Monograph during the course of the study
4. Relevant curriculum vitae
5. Human Subjects Training Certification
6. Any specific information the review board requires.

The Investigator must provide the following documentation to the CCC:

1. The institutional review board's initial and annual re-approval of the protocol.
2. The institutional review board's approvals of any revisions of the informed consent document or amendments to the protocol or informed consent.

11.2 Subject Confidentiality

All laboratory specimens, evaluation forms, reports, video recordings, and other records that leave the site will be identified only by the Study Identification Number (SID) to maintain subject confidentiality. All records will be kept in a locked file cabinet. All computer entry and networking programs will be done using SIDs only. Clinical information will not be released without written permission of the subject, except as necessary for monitoring by IRB, the FDA, the NINDS, the OHRP, the Sponsor, or the Sponsor's designee.

Radiographic Masking: Although determinations for routine patient care will be performed locally, radiographic determination needed for treatment comparisons will be made in a central radiological setting and be part of the permanent data files. Centralizing the CT and MRI scan interpretations ensures that the required masks are maintained. Central reading will assure a high degree of uniformity and standardization of the measurement of the hemorrhage size and assessment of edema and mass effect. The central radiologist and radiology technician will be blinded to clinical information such as the response of the patient to MIS with or without rt-PA. All imaging studies will be catalogued and analyzed, and the results entered into the EDC system.

11.3 Study Modification/Discontinuation

The study may be modified or discontinued at any time by the IRB, the NINDS, the Sponsor, the OHRP, the FDA, or other government agencies as part of their duties to ensure that research subjects are protected.

12 PUBLICATION OF RESEARCH FINDINGS

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

The results of the trial will be published regardless of its outcome. A Publication Committee will be established. Publication regarding further analyses performed on the data will be by mutual agreement between the EC and the site investigators.

The investigator may publish or present at scientific meetings the results of this study, provided that confidential information is not disclosed, and only after obtaining advance written consent from the EC. Consent may be withheld at the sole discretion of Executive Committee.

In this regard, a copy of all public disclosures, including but not limited to publication manuscripts, abstracts, and seminar presentations, should be provided to the EC for

review, at least 30 days before the manuscript is submitted to the publisher or a presentation is made.

Additionally, the Clinical Study Report (final study report) and any literature articles that are a result of the study should be sent to Genentech. Copies of such reports will be faxed to the assigned Clinical Operations Contact for the study: Lytics IST central mailbox: lytics-gsur@gene.com or fax: 866-283-2263.

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Appendix 1: Sample trial consent form

RESEARCH PARTICIPANT INFORMED CONSENT AND PRIVACY AUTHORIZATION FORM

Protocol Title: MISTIE III: A phase III, randomized, open-label, 500-subject clinical trial of minimally invasive surgery plus rt-PA in the treatment of intracerebral hemorrhage.

[Add only if your site is participating in the ancillary study.]
MTI-M3: Mechanisms of Tissue Injury in MISTIE III

Protocol No.: Sponsor protocol number: ICH02

Sponsor: Daniel F. Hanley, MD
Director, Brain Injury Outcomes Division
Johns Hopkins University Department of Neurology
Baltimore, Maryland, USA

Primary funding: National Institutes of Health/
National Institute of Neurological Disorders and Stroke

Additional support: [US and Canadian sites: Genentech, Inc.]
[Non-US and non-Canadian sites: National Institute for Health Research]

Principal Investigator:

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1. What you should know about this study:

- You are being asked to join a research study.
- This consent form explains the research study and your part in the study.
- Please read it carefully and take as much time as you need.
- Please ask questions at any time about anything you do not understand.
- You are a volunteer. If you join the study, you can change your mind later. You can decide not to take part or you can quit at any time. There will be no penalty or loss of benefits if you decide to quit the study.
- During the study, we will tell you if we learn any new information that might affect whether you wish to continue to be in the study.
- Ask your study doctor or the study team to explain any words or information in this informed consent that you do not understand.

- A description of the research will be available at www.ClinicalTrials.gov. This website will not include information that can identify you. You can search the website at any time.
- The person being asked to be in this research study may not be able to give consent to be in this study. You are therefore being asked to give permission for this person to be in the study as his/her decision maker.
- [Add only if your site is participating in the ancillary study.] Biospecimens will be collected in this study. These biospecimens may include blood samples as well as samples of fluid that would otherwise be discarded. This may include fluid collected from the hematoma at surgery or from the drain placed within the hematoma as part of the MISTIE trial. Most biospecimens contain DNA, which is the genetic code for each person.

2. Why is this research being done?

This research is being done to evaluate the recovery of participants who receive a study drug called rt-PA (recombinant tissue plasminogen activator) when used with minimal surgery for the removal of a blood clot from the brain compared to participants who receive standard medical care (in other words, no surgery and no study drug). [Add only if your site is participating in the ancillary study.] Additionally, this research will help to determine if radiographic images, genetic data and markers of inflammation in the brain collected from blood and brain fluid samples can help to identify those patients with brain hemorrhage who will most likely benefit from the minimal surgery.

People with certain types of bleeding in the brain may join. This includes bleeding that happened without warning and which is not caused by a head injury. This unexpected bleeding is called ICH or intracerebral hemorrhage. ICH typically occurs in patients with high blood pressure or in the elderly due to fragile blood vessels.

The rate of death in patients with ICH is still very high despite the best medical treatment. Also, the amount of recovery in those that survive is also very poor. It has been shown that the amount and success of recovery is related to the size of the blood clot in the head. However, extensive surgery to remove the blood clot has sometimes been shown to be more harmful. Therefore, the usual treatment for ICH is to avoid doing extensive surgery whenever possible. This usual treatment is called “standard medical care.”

Recent studies have shown that a less aggressive method of removing the blood clot - by using a small drain tube surgically placed into the brain to give a medicine to break up the clot - can be of benefit. This study will allow us to see if this method of clot evacuation is more effective at improving recovery than standard medical care, which does not involve removing the clot.

Rt-PA is approved by the U.S.A. Food and Drug Administration (FDA) and the European Medicines Agency (EMA) for the treatment of heart attacks or ischemic stroke (clot in

the brain) for dissolving clots. It is not approved for use in hemorrhagic stroke (bleeding in the brain) The FDA is allowing the use of rt-PA in this study.

How many people will be in this study?

About 500 participants total are expected to take part in this research study, with about 5-10 subjects from [Your Institution Name].

3. What will happen if you join this study?

If you agree to be in this study, we will ask you to do the following things:

Screening: With your consent, you will have the following screening procedures to find out if you are eligible for this study:

- A CT scan will be done at least 6 hours after the first CT scan that diagnosed the bleeding in your brain. If this second CT scan shows more blood in the brain when compared to the first CT scan, another CT scan will be repeated at least 6 hours later. This is done to make sure the bleeding has stopped. A CT scan is a test that produces an image of your body using a small amount of radiation. The image shows the body tissues and structure in three dimensions ("3-D"). These CT scans may already have been done per standard clinical care.

- A MRI (magnetic resonance imaging) scan and a MRA (magnetic resonance angiogram) or a CTA (computed tomography angiogram) will be done. These procedures are part of the standard clinical care to see if the bleeding is caused by abnormal blood vessels, such as an aneurysm. You will not be eligible for this study if this is the cause for your bleeding.

- A pregnancy test will be done if you are a female of childbearing potential. You must not be pregnant to be in this study. This test is part of the standard clinical care.

Randomization: If, after these tests, you are found to be eligible for this study, you will be randomly assigned (50%-50% chance, similar to flipping a coin) to one of the two methods being compared in this study. You will either continue to receive standard medical care, or a small tube will be surgically placed into the clot to allow it to drain. You will have 1 chance out of 2 to be selected to have surgery. The drug rt-PA may be given through this tube to help break-up the clot if the drain alone does not remove enough of it.

Standard medical care: If you are randomly assigned to continue to receive standard medical care:

- Your vital signs, such as, heart rate, blood pressure, temperature, and neurological condition will be monitored daily for the next 6 days. This is done as part of standard clinical care but will be reviewed for study purposes.
- Blood samples, about 3 to 4 teaspoons, will be drawn daily for the next 6 days to monitor natural chemical levels in the blood related to the bleeding in your brain. This is done as part of standard clinical care but will be reviewed for study purposes.
- CT scans will be done daily for the next 4 to 5 days to monitor the remaining blood clot in your brain. This is done as part of standard clinical care but will be reviewed for study purposes.
- An MRI will be performed at day 7. This is done for study purposes only and only if you are able to have an MRI (see Risks section below). At some point during the MRI exam, the scanning procedure will be interrupted to give you a contrast agent through a needle in your arm.
- [Add only if your site is participating in the ancillary study.] Blood will be drawn (2 teaspoons each time) from a needle in the arm or from an intra-arterial or intravenous line once daily for 5 days. This will be done at the same time as other routine blood collection and using the same sterile technique for usual blood draws.
- [Add only if your site is participating in the ancillary study.] Blood will be drawn (four teaspoons) once while you are in the hospital or during a scheduled follow-up visit.

Surgery plus study drug: If you are randomly chosen or assigned to the group that will get the drain:

- You will be taken to an operating room or other designated area and given an appropriate, general anesthetic. A neurosurgeon will make a skin incision over the site of the blood clot. Following this, a hole will be drilled in the skull through the skin opening and an unbendable, hollow tube will be passed into the clot. When the tube is in the right place, suction will be applied to the drain using a syringe to remove as much of the blood clot as possible. A soft rubber drain tube (called a catheter) will be passed through the tube and the unbendable tube will be removed. The soft rubber drain tube will be left in the clot in the head and the skin will be closed around it.
- Another CT scan will then be done to see how much clot is left and to make sure that the soft drain tube is in the middle of the remaining blood clot.
- You will then be taken to the intensive care unit.
- If there is enough blood clot remaining in the brain after the surgery, rt-PA (a drug that breaks up blood clots) along with a saline (salt water) fluid (to flush the tubing) will be given into the drain every 8 hours to break up the clot. In-between injections, the drain tube will be attached to a drainage system to allow the clot to come out on its own.

- Once a day you will be taken to have another CT scan. Injections of rt-PA will stop after 9 doses have been given or when enough blood has been removed from the clot, whichever comes first.
- Your vital signs, such as, heart rate, blood pressure, temperature, and neurological condition will be monitored daily for the next 6 days. This is done as part of standard clinical care but will be reviewed for study purposes.
- Blood samples, about 3 to 4 teaspoons, will be drawn for the next 6 days to monitor natural chemical levels in the blood related to the bleeding in your brain. This is done as part of standard clinical care but will be reviewed for study purposes.
- CT scans will be done daily for the next 4 to 5 days to monitor the remaining blood clot in your brain. This is done as part of standard clinical care but will be reviewed for study purposes.
- An MRI will be performed at day 7. This is done for study purposes only and only if you are able to have an MRI (see Risks section below). At some point during the MRI exam, the scanning procedure will be interrupted to give you a contrast agent through a needle in your arm.
- [Add only if your site is participating in the ancillary study.] Blood will be drawn (four teaspoons) once while you are in the hospital or during a scheduled follow-up visit.
- [Add only if your site is participating in the ancillary study.] Blood will be drawn (2 teaspoons each time) from an intravenous line once daily for 5 days. This will be done at the same time as other routine blood collection and using the same sterile technique for usual blood draws.
- [Add only if your site is participating in the ancillary study.] The blood clot removed during the placement of the brain catheter will be collected.
- [Add only if your site is participating in the ancillary study.] Blood clot drainage will be collected (2 teaspoons) from the drainage bag/chamber once daily while the brain catheter is in place. This will be done using sterile technique.

[Add this section only if your site is participating in the ancillary study.]
Genetic and Inflammatory Marker Testing

- We will prepare the blood collected for genetic and inflammatory marker testing and analysis. The study staff will send the blood samples to be stored with specific investigators for 10 years. You will have the choice to allow your stored blood samples to be used in future related studies for brain bleeding strokes.
- The Genetic Information Nondiscrimination Act (GINA) may help protect you from health insurance employment discrimination based on genetic information.
- The law provides that health insurance companies and group health plans
    - May not ask for genetic information from this research and
    - May not use genetic information when making decision about eligibility or premiums

  - The law will not stop health insurance companies from using genetic information to decide whether to pay claims. The law does not apply to other types of insurance (such as life, disability or long-term care).
- Request to collect and store biospecimens for future research
  - As part of this research study, we would like to ask you to let us store your biospecimens and health information for future research. This research could include other diseases and involve research tools such as gene sequencing or the creation of cell lines.
  - Gene sequencing of your DNA provides researchers with the code to your genetic material.
  - Cell lines are living tissue samples that can be grown in a laboratory. A cell line can provide an unlimited supply of cells in the future without requiring more samples from you. Each cell contains your complete DNA.
  - The study doctor can provide you with additional information if you have questions. Also, further information about our use of your biospecimens can be found in this consent document under the heading *What happens to Data and Biospeciments that are collected in the study?*
  - Will you allow us to store the biospecimens we collect for this study for use in future research?

    YES _____
    Signature of Participant

    NO _____
    Signature of Participant

Study follow visits: Study participants will be followed for 1 year after randomization. Regardless of what group you are assigned to:

- You will be asked to return to the clinic 30, 180, and 365 days from today. Your neurological condition and blood pressure will be checked and you will be asked questions about how well you are doing. These visits will take about 2 hours and will be video recorded. This video will be sent to an expert doctor in the United Kingdom for review. If during the 30-, 180- and 365-day follow-up visit, we are unable to interview you directly, we will ask permission to interview the person accompanying you during the visit. There will be a separate consent form for the person accompanying you to sign at that time, if it is necessary.
- During the clinic visits at 30 and 180 days from today you will have CT scan to look at how your brain is healing.
- You will be contacted by telephone 90 and 270 days from today. You will be asked questions about your condition and how well you are doing.

How long will you be in the study?

You will be in this study for 12 months.

4. What are the risks or discomforts of the study?

STANDARD MEDICAL CARE GROUP:

Likely risks:

- Approximately 70% of ICH patients receiving standard medical care will normally have further bleeding in the brain. This is called rebleeding. We will review daily CT scans to watch for rebleeding. We will also review daily blood tests to watch for bleeding disorders.
- You will have daily CT scans. The radiation exposure from the CT scans you will receive by participating in this study is equivalent to an exposure of 1.4 rems (14 mSv) to your whole body. For comparison, naturally occurring radiation from the environment exposes people to about 0.3 rems (3 mSv) per year and people exposed to radiation in their occupations are permitted to receive whole body exposures of 5 rems (50 mSv) per year.

Less likely risks:

- Approximately 10% to 20% of ICH patients receiving standard medical care will normally develop infection. If you develop signs and symptoms of infection, your clinical care team will take samples of your spinal fluid to look for infection if necessary.
- Worsening of your neurological condition.
- The effects of magnetic fields in an MRI scanner have been extensively studied, and there are no known significant risks with an MRI exam. You may, however, be bothered by feelings of confinement (claustrophobia), and by the noise made by the magnet during the procedure. You will be asked to wear earplugs or earphones while in the magnet. You will not have an MRI if you have a pacemaker, an implanted defibrillator or certain other implanted electronic or metallic devices. It is important for you to advise the MRI staff if you have had brain surgery for a cerebral aneurysm, or if you have implanted medical or metallic devices, shrapnel, or other metal, such as metal in your eye.
- The contrast agent you will receive is FDA-approved and used routinely for MRI exams. It contains a material called gadolinium. About 1 in 100 people may notice discomfort, tingling or warmth in the lips, metallic taste in the mouth, tingling in the arm, nausea, or headache. These symptoms go away quickly. There is a small risk of an allergic reaction to gadolinium. However, a severe allergic reaction occurs in less than one in 300,000 people. The placement of the needle (small plastic tube) to give you the gadolinium may cause minor pain, bruising and/or infection at the injection site.
- People with severe kidney failure who receive gadolinium are at risk of developing Nephrogenic Systemic Fibrosis/Nephrogenic Fibrosing Dermopathy (NSF/NFD). This disease causes fibrosis (the formation of too

much connective tissue in the skin and internal organs). This is a serious disease which can result in death. You should notify the study team or MRI staff if you are allergic to gadolinium or if you have kidney problems.

- Drawing blood from your arm may cause pain, bruising, lightheadedness, and, on rare occasions, infection.
- **[Add only if your site is participating in the ancillary study.]** Despite the GINA protections and the best efforts of the research team, there may still be a risk if information about you were to become known to people outside of this study.
- **[Add only if your site is participating in the ancillary study.]** Genetic information is unique to you, even without your name or other identifiers. For this reason, genetic information like DNA may be used to identify you and possibly your family members. We have procedures (such as, labeling your biospecimens with a password protected code known only to select research staff) to prevent people working with your DNA from discovering if it belongs to you. However, there is the risk this can happen as new ways of tracing genetic information are being developed that may make re-identification of genetic information possible.
- Death.

Discomforts:

- You may experience discomfort that is part of the routine medical care for participants with your condition in the intensive care unit.
- During the follow up visits, you may get tired or bored when we are asking you questions or you are completing questionnaires. You do not have to answer any question you do not want to answer.

There may be side effects and discomforts that are not yet known.

SURGERY PLUS STUDY DRUG GROUP:

Likely risks:

- In this study, the risk of rebleeding could be higher than normal. Certain procedures such as inserting the drain tube into the clot, injecting the clot-dissolving drug, and removing the drain tube may increase the risk. We will review daily CT scans to watch for rebleeding. We will also review daily blood tests to watch for bleeding disorders. If you have rebleeding in the brain that causes a worsening of your neurological condition, we will stop injecting the study drug.
- You will have daily CT scans. The radiation exposure from the CT scans you will receive by participating in this study is equivalent to an exposure of 1.4 rems (14 mSv) to your whole body. For comparison, naturally occurring radiation from the environment exposes people to about 0.3 rems (3 mSv) per year and people exposed to radiation in their occupations are permitted to receive whole body exposures of 5 rems (50 mSv) per year.

Less likely risks:

- In this study, the injection of the study drug (rt-PA) could increase the risk of infection. If you develop signs and symptoms of infection, your clinical care team will take samples of your spinal fluid to look for infection if necessary.
- The placement of the drain tube and leaving it in place for 3 days may further increase the risk of infection.
- We do not yet know if your overall risk is higher or lower if you get the drain tube and the study drug.
- Worsening of your neurological condition.
- The effects of magnetic fields in an MRI scanner have been extensively studied, and there are no known significant risks with an MRI exam. You may, however, be bothered by feelings of confinement (claustrophobia), and by the noise made by the magnet during the procedure. You will be asked to wear earplugs or earphones while in the magnet. You will not have an MRI if you have a pacemaker, an implanted defibrillator or certain other implanted electronic or metallic devices. It is important for you to advise the MRI staff if you have had brain surgery for a cerebral aneurysm, or if you have implanted medical or metallic devices, shrapnel, or other metal, such as metal in your eye.
- The contrast agent you will receive is FDA-approved and used routinely for MRI exams. It contains a material called gadolinium. About 1 in 100 people may notice discomfort, tingling or warmth in the lips, metallic taste in the mouth, tingling in the arm, nausea, or headache. These symptoms go away quickly. There is a small risk of an allergic reaction to gadolinium. However, a severe allergic reaction occurs in less than one in 300,000 people. The placement of the needle (small plastic tube) to give you the gadolinium may cause minor pain, bruising and/or infection at the injection site.
- People with severe kidney failure who receive gadolinium are at risk of developing Nephrogenic Systemic Fibrosis/Nephrogenic Fibrosing Dermopathy (NSF/NFD). This disease causes fibrosis (the formation of too much connective tissue in the skin and internal organs). This is a serious disease which can result in death. You should notify the study team or MRI staff if you are allergic to gadolinium or if you have kidney problems.
- Drawing blood from your arm may cause pain, bruising, lightheadedness, and, on rare occasions, infection.
- **[Add only if your site is participating in the ancillary study.]** The removal of hematoma drainage may be associated with a small increased risk of infection in the brain. We will minimize the risk of infection by using a standardized sterile technique to remove the hematoma drainage.
- **[Add only if your site is participating in the ancillary study.]** Despite the GINA protections and the best efforts of the research team, there may still be a risk if information about you were to become known to people outside of this study.

- **[Add only if your site is participating in the ancillary study.]** Genetic information is unique to you, even without your name or other identifiers. For this reason, genetic information like DNA may be used to identify you and possibly your family members. We have procedures (such as, labeling your biospecimens with a password protected code known only to select research staff) to prevent people working with your DNA from discovering if it belongs to you. However, there is the risk this can happen as new ways of tracing genetic information are being developed that may make re-identification of genetic information possible.
- Death.

Discomforts:

- You will have to stay in bed while the drain tube is in place.
- You may experience discomfort that is part of the routine medical care for participants with your condition in the intensive care unit.
- During the follow up visits, you may get tired or bored when we are asking you questions or you are completing questionnaires. You do not have to answer any question you do not want to answer.

There may be side effects and discomforts that are not yet known.

5. Are there risks related to pregnancy?

Because of the need for head CT and MRI scans, you will have a pregnancy test if you are a female of childbearing potential. You may not take part in this study if you are pregnant or nursing.

If you become pregnant during the 12 month follow up period, we will ask you to complete the interviews and questionnaires, but you will not have the CT scans done.

This research may hurt an embryo or fetus in ways we do not currently know.

6. Are there benefits to being in the study?

Your intracerebral hemorrhage (ICH) may improve while you are in this study; however, this cannot be promised. The results of this study may help people with ICH in the future. There is no guarantee that you will receive any medical benefits from being in this study.

There may be no benefit to you from use of the study drug. We hope to show that rt-PA in combination with minimal surgery, will decrease the size of the blood clot in your head allowing you to recover faster. If you are assigned to receive standard medical care, you may not have this benefit. If this study shows that the use of rt-PA in combination with minimal surgery is more effective than medical treatment, it could be of benefit to many more patients who have bleeding into the brain.

It cannot be promised that you will receive any medical benefits from being in this study.

7. What are your options if you do not want to be in the study?

If you decide not to join this study, there are no other specific treatments available. Currently the only alternative to this treatment is standard medical management without removal of the blood clot. Ask the study doctor to discuss possible medical and surgical options with you.

You do not have to join this study. If you do not join, your care at [Your Institution Name] will not be affected.

8. Will it cost you anything to be in this study?

The following procedures, tests, drugs or devices are part of this research study and will be supplied free of charge by the study:

- Pregnancy test (if a second test is done post-consent)
- Activase administrations (via intraclot catheter; surgical group only)
- ICU interviews and clinic visits to evaluate neurological function and your recovery

[US and Canadian Sites: This study and the procedures listed below qualify under CMS rules for Medicare/Medicaid coverage. Most private insurers follow CMS rules for coverage of procedures related to being in a clinical study. Your health insurer will be responsible for all other procedures, tests, drugs, or devices that are part of this study such as the following:]

[Non-US and non-Canadian Sites: All patients are insured according to the Medicines Act. Your health insurer will be responsible for all other procedures, tests, drugs, or devices that are part of this study such as the following:]

- Diagnostic CT
- Stability CT (6 hours or more after diagnostic CT)
- Blood pressure control sustained over a minimum of 6 hours
- Pregnancy test
- MRI/MRA and CTA
- Image-Guided Catheter Placement + Aspiration (Surgical Group Only)
- Post catheter placement CT scan
- Daily CT scan
- CT obtained 24 h post catheter removal
- Neurocheck
- Lab Assessments- Coagulation tests: PT, aPTT, INR
- Lab Assessments- CBC including platelet counts
- Lab Assessments- Chemistries

[US and Canadian sites: If an insurer denies coverage for any of the above listed procedures, the study will pay for the denied claim. If you have private health insurance, you will be responsible for deductibles. If there are co-pays *for study related charges*, the study will cover the co-pays. If you have received a medical bill related to this research participation, please contact [Study Coordinator Name] at [Study Coordinator Phone Number].

Funds for MISTIE III are provided by the National Institutes of Health (NIH), National Institute for Neurologic Disorders and Stroke (NINDS). These funds are not available to cover the costs of any other medical care, and you are responsible for the cost of your hospitalization and care not related to research. If for some reason your insurance denies the claim for your hospital bill, there may be services available to you, if you choose, to help you through the claims appeal process. If you have questions about your medical bill not relative to research participation, you may contact [Study Coordinator Name] at [Study Coordinator Phone Number], who can refer you to hospital sources that may help with your hospital charges.]

[Non-US and non-Canadian sites: If you have received a medical bill related to this research participation, please contact [Study Coordinator Name] at [Study Coordinator Phone Number]. [Add country-specific insurance information]

9. Will you be paid if you join this study?

You will not be paid for participation in the study. The study will reimburse $50 per visit for travel expenses as part of the 30, 180, and 365 day follow-up visits only. All other visits will take place while you are in the hospital or over the telephone.

10. Can you leave the study early?

- You can agree to be in the study now and change your mind later.
- If you wish to stop, please tell us right away.
- Leaving this study early will not stop you from getting regular medical care.
- If you leave the study early, [Your Institution Name] may use or give out your health information that it already has if the information is needed for this study or any follow-up activities.

11. Why might we take you out of the study early?

You may be taken out of the study if:

- Staying in the study would be harmful.
- You need treatment not allowed in the study.
- The study is cancelled.

- There may be other reasons to take you out of the study that we do not know at this time.

If you are taken out of the study early, [Your Institution Name] may use or give out your health information that it already has if the information is needed for this study or any follow-up activities.

12. How will your privacy be protected?

[US and Canadian sites: [Your Institution Name] has rules to protect information about you. Federal and state laws also protect your privacy. This part of the consent form tells you what information about you may be collected in this study and who might see it.

Generally, only people on the research team will know that you are in the research study and will see your information. However, there are a few exceptions that are listed later in this section of the consent form.

The people working on the study will collect information about you. This includes things learned from the procedures described in the consent form. They may collect other information including your name, address, date of birth, and other details.

The research team will need to see your information. Sometimes other people at [Your Institution Name] may see or give out your information. These include people who review the research studies, their staff, lawyers, or other [Your Institution Name] staff.

People outside of [Your Institution Name] may need to see your information for this study. Examples include government groups (such as the Food and Drug Administration), safety monitors, other hospitals in the study and companies that sponsor the study.

We cannot do this study without your permission to use and give your information. You do not have to give us this permission. If you do not, then you may not join this study.

We will use and disclose your information only as described in this form and in our Notice of Privacy Practices; however, people outside of [Your Institution Name] who receive your information may not be covered by this promise. We try to make sure that everyone who needs to see your information keeps it confidential – but we cannot guarantee this.

The use and disclosure of your information has no time limit. You can cancel your permission to use and disclose your information at any time by contacting the Principal Investigator of this study. The Principal Investigator can be reached by phone at [Phone Number] or by sending a letter to:

[PI name]
[PI address]

[PI fax number]

You may also choose the option of contacting the [Your Institution Name] Privacy Officer. The [Your Institution Name] Privacy Officer can be reached by phone at [Phone Number] or by sending a letter to:

[Your Institution Name] Privacy Officer
[Address]
[Fax number]

If you send a letter, please be sure to include the study number and your contact information.

If you do cancel your permission to use and disclose your information, your part in this study will end and no further information about you will be collected. Your cancellation would not affect information already collected in this study.]

[Non-US and non-Canadian sites: Insert country-specific privacy language.]

13. Will the study require any of your health care providers to share your health information with the researchers of this study?

As part of this study, the researchers may ask to see your health care records from your other health care providers.

14. What treatment costs will be paid if you are injured in this study?

The costs for any treatment or hospital care you received as the result of a study-related injury will be billed to your health insurer. Any costs that are not paid for by your health insurer will be billed to you.

15. What other things should you know about this research study?

a. What is the Institutional Review Board (IRB) [Ethics Committee (EC)] and how does it protect you?

The [Your Institution Name] IRB is made up of:

- Doctors
- Nurses
- Ethicists
- Non-scientists
- and people from the local community.

The IRB [EC] reviews human research studies. It protects the rights and welfare of the people taking part in those studies. You may contact the IRB [EC] if you have questions about your rights as a participant or if you think you have not been treated fairly. The

IRB [EC] office number is [Phone Number]. You may also call this number for other questions, concerns or complaints about the research.

b. What do you do if you have questions about the study?
Call the principal investigator, Dr. _____ at [Phone Number]. If you wish, you may contact the principal investigator by letter at [Address] or by fax at [Fax Number]. If you cannot reach the principal investigator or wish to talk to someone else, call the IRB [EC] office at [Phone Number].

c. What should you do if you are injured or ill as a result of being in this study?
Call Dr. _____ at [24 hour Phone or Pager Number] if you have an urgent medical problem related to your taking part in this study.

If this number is a pager number, after the tone, enter the phone number where you can be called, press the # key, and hang up.

d. What happens to Data, Imaging (CT, MRI, etc.) scan copies, and biospecimens that are collected in the study?
The data, imaging scan copies, and biospecimens collected from you during the study are important to both this study and to future research.

If you join this study:
- You will not own the data or copies of imaging scans given by you to the investigators for this research.
- Both [Your Institution Name] and any Sponsor of this research may study your data and imaging scan copies collected from you.
- If data or imaging scan copies are in a form that identifies you, [Your Institution Name] may use them for future research only with your consent or IRB [EC] approval.
- All biospecimens will be stored at [Your Institution Name] before secure transfer to one or more of the central study laboratories for analysis: University of Manchester, UK, Massachusetts General Hospital, Boston, USA, and/or Yale University, New Haven, Connecticut, USA.
- You will not own any product or idea created by the researchers working on this study.
- You will not receive any financial benefit from the creation, use or sale of such product or idea.

Often it is helpful for scientists to share information they get from studies in order to learn more about health and disease. Combining information from different studies in one place may help them learn even more. This collection of information is called a databank. Your study data may be sent to one or more databanks, where it will be stored with data from other studies. The databanks may be kept at universities, government agencies (such as the National Institutes of Health), or private companies. The data may include health

information and images (for example, X-rays, MRIs, or CT scans). Results of this study will also be published in a medical journal(s).

Your name and any other identifying information will NEVER be included in information that is published in a medical journal(s) or sent to a databank(s) (this is called de-identification). Researchers will ALWAYS have a duty to keep your information confidential.]

e. What are the Organizations that are part of [Your Institution Name]?
[Your Institution Name] includes the following:
- [List out any other names or locations that serve your institution]

16. What does your signature on this consent form mean?

Your signature on this form means that:
- you understand the information given to you in this form
- you accept the provisions in the form
- you agree to join the study

You will not give up any legal rights by signing this consent form.

WE WILL GIVE YOU A COPY OF THIS SIGNED AND DATED CONSENT FORM

Signature of Participant	Date/Time
Signature of Person Obtaining Consent	Date/Time
Signature of Legally Authorized Representative (LAR) for **ADULTS NOT CAPABLE of GIVING CONSENT** *(Persons from the following categories in order of priority may be a Legally Authorized Representative: Health Care Agent; Legal Guardian; Spouse; Adult child; Parent; Adult sibling; Friend or other relative)*	Date/Time
Relationship of LAR to Participant (indicate why the LAR is authorized to act as a surrogate health care decision-maker under state law)	Date/Time
Signature of Witness to Consent Procedures (optional unless IRB or Sponsor required)	Date/Time

NOTE: A COPY OF THE SIGNED, DATED CONSENT FORM MUST BE KEPT BY THE PRINCIPAL INVESTIGATOR; A COPY MUST BE GIVEN TO THE PARTICIPANT; AND, IF

APPROPRIATE A COPY OF THE CONSENT FORM MUST BE PLACED IN THE PARTICIPANT'S MEDICAL RECORD.

ONLY CONSENT FORMS THAT INCLUDE THE [Your Institution Name] LOGO CAN BE USED FOR CONSENTING RESEARCH PARTICIPANTS. IF THIS CONSENT FORM DOES NOT HAVE A [Your Institution Name] LOGO, DO NOT USE IT TO CONSENT RESEARCH PARTICIPANTS.

Appendix 2: Sample HIPAA authorization form for international research

HIPAA Authorization Form for International Research

Principal Investigator:

Application Number:

Study Title: MISTIE III: A phase III, randomized, open-label, 500-subject clinical trial of minimally invasive surgery plus rt-PA in the treatment of intracerebral hemorrhage.

Some of your health information collected in this study will be sent to the United States. The U.S. has privacy laws that will protect your information and your identity.

If you want to be in the study, you must agree to let us use and send details about you and your health as part of this study. This study uses a drug. The U.S. Food and Drug Administration (FDA) may need to see your health information when it is sent to the U.S.

If you join the study, you can decide later that you want to leave the study and you do not want to have your health information sent to the U.S. **If you decide to leave the study, we will not be able to take back any health information that has already been sent to the U.S.** To leave the study, tell the principal investigator.

Please sign this form (or make your mark) if you agree to let us use and give out details about your health.

Signature of Participant	Date/Time
Signature of Person Obtaining Consent	Date/Time
Signature of Legally Authorized Representative (LAR) for **ADULTS NOT CAPABLE of GIVING CONSENT** *(Persons from the following categories in order of priority may be a Legally Authorized Representative: Health Care Agent; Legal Guardian; Spouse; Adult child; Parent; Adult sibling; Friend or other relative)*	Date/Time
Relationship of LAR to Participant (indicate why the LAR is authorized to act as a surrogate health care decision-maker under state law)	Date/Time

(This form is to be kept with the consent document signed by the study participant or LAR.)

Appendix 3: Medical management guidelines

1. ICP management: The American Academy of Neurological Surgery's (AANS) Head Injury Guidelines will be used as the standardized approach for both the medical and surgical treatment groups. This approach has been previously employed. (Clifton GL, Miller ER, Choi SC, et al: Lack of effect of induction of hypothermia after acute brain injury. N Engl J Med 344:556-563, 2001) Intracranial hypertension is defined as pressure within the cranial vault elevated $\geq$ 30 mm Hg for five or more minutes. A patient will be monitored if he or she demonstrates obtundation, which we define as GCS $\leq$ 8 on a minimum of two observations over eight hours of time. All eligible patients will be monitored, independent of medical or surgical treatment. The treating surgeon will use standardized clinical criteria to select the ICP monitoring device. The Camino parenchymal catheter has been pre-specified as the device of choice. However the intraventricular catheter could be a choice, if it offers clinical advantage in the presence of non-compressed ventricles, or a subarachnoid screw could be chosen, if there is risk of infection.

Interventions include: 1) head positioning (usually 30^{o} elevation HOB), 2) euthermia with core temperature $\leq 38^{o}$, 3) normoxia and normocapnia, and 4) sedation and analgesia, to maintain HR $\leq$ 120 with concurrent absence of agitated motor activity. When standard interventions are not effective, mannitol in doses of 1 gm/kg load and 0.25 gm per kg maintenance will be administered. In response to acute sustained ICP elevation ($\geq$ 40 mm Hg or refractory ICP elevation), hyperventilation to a $PaCO_2 \leq 25$ mm Hg will be performed. Ventilation parameters, including F_iO_2 and tidal volume, respiratory rate, and ventilation mode, will be set to produce $SaO_2 > 90\%$ saturation and mean airway pressures $\leq$ 20 cm H_2O. Prolonged sedation with propofol will be used for transient or sustained ICP $\geq$ 30 mm Hg, where agitation is deemed a possible factor. Surgical management of uncontrollable ICP to control ICP is allowed but not encouraged in the absence of full medical therapy. Surgery may be considered if hemorrhage extension or rebleeding occurs, if ICP $\geq$ 30 mmHg, with optimal medical management, for acute compartment syndrome, or other life-saving consideration. When ICP is controlled at $\leq$ 20 mm Hg for one or more days, sequentially withdraw treatment modalities from highest level of intervention to lowest level of intervention. Every effort will be made to avoid long term hyperventilation, in keeping with the AANS head injury guidelines (American Association of NS: Guidelines for the management of severe head injury. Brain Trauma Foundation, American Association of Neurological Surgeons, Joint Section on Neurotrauma and Critical Care. J Neurotrauma 13:641-734, 1996) and the ASA ICH treatment guideline statement. (Broderick JP, Connolly S, Feldman E: Guidelines for the management of spontaneous intracerebral hemorrhage: a statement for healthcare professionals from a special writing group of the Stroke Council, American Heart Association. Stroke 38:2001-2023, 2007)

ICP monitoring devices will be removed when ICP is maintained < 20 mm Hg without pharmacological therapy.

2. Neurological status: The GCS goal is 15 or 10T sustained for 8 hours of observation. Sedation will be used for agitation. The sedations of choice are Propofol or Lorazepam

0.5 mg, IV, Q1-2 hr., to maintain a sedation score of 1. Sedation will be discontinued when ICP is successfully controlled.

3. Cardiovascular management: Beta-blockade with Metoprolol, 20 to 80 mg IV Q8 hrs, will be the primary therapeutic modality. Transient elevations of BP > 160 mm Hg will be treated with labetalol 5-10 mg IV. A second agent will be initiated where coronary artery disease is suspected by EKG or by historical criteria. Where renovascular hypertension appears likely, the angiotension converting enzyme inhibitor enalapril will be administered. When necessary for sustained BP control, a constant infusion of esmolol will be the first line drug, heart rate permitting (HR $\geq$ 90 beats per min).

4. Respiratory care: A trial of independent breathing will be undertaken and/or the level of mechanical ventilatory support will be diminished, when no pooled secretions exist, the LOC is $\geq$ 10 GCS points, and oxygenation is sustained. The absence of ongoing ICP elevation and the presence of independent sustained mechanical ventilatory activity for > 12 hours will be considered sufficient criteria to consider removal of the endotracheal tube in all patients with intact airway reflexes.

5. Nutritional support: Ranitidine and/or reglan may be used to suppress gastric acid. For patients with persistent ileus, after pharmacologic motility enhancement for > 48 hours, a trial of parenteral nutrition will be undertaken. The presence of established independent ventilation and the absence of aspiration on bedside swallowing tests will be considered the necessary prerequisites for a trial of oral feeding. Enteral or parenteral feeds will not be discontinued until a minimum of 80% of daily caloric needs is consistently met by oral intake.

6. Thromboembolic prophylaxis: Deep venous thrombophlebitis and pulmonary embolus prophylaxis will be undertaken on the day of admission with the use of SCDs. For patients at high risk of thromboembolism, study center standard of care policies may govern the use of low molecular weight, fractionated and unfractionated heparins for DVT prophylaxis during the acute treatment and follow-up periods (criteria established by the American Orthopedic Association).

7. Withdrawal of care: Withdrawal of care discussions of prognosis and decisions to continue or limit, or to withdraw, life-sustaining interventions will be conducted according to each institution's policies for end-of-life decision-making, as well as their institutional codes of medical ethics. The study assumes any such discussion will reflect the patient's wishes and the known facts regarding prognosis. Where the PI is not the managing physician it is assumed that those individuals will confer prior to presentation of the consensus prognosis and planned course of treatment. In some situations, the investigator may choose to select a colleague to serve in the clinician role or request a review by the hospital's ethics committee or other knowledgeable expert.

Appendix 4: Sample consent form for videotaping a proxy during the modified Rankin Scale interview.

MISTIE III Proxy Videotaping Consent

The purpose of this document is to obtain your consent to talk with you on videotape

1. In the MISTIE III study, we record each patient at three follow-up visits
2. Today, we are unable to record an interview with the patient who is enrolled in the MISTIE III study but is too ill to speak
3. We would like to record you instead while you briefly describe the patient's condition
4. The purpose of the recording is to have the same central readers at the University of Glasgow evaluate every patient's condition
5. Only physicians and staff at the University of Glasgow will see the video in a professional hospital research setting
6. The recording will not be broadcast or used for any other purpose and no other copies will be made
7. The recording may be stored on a computer server for 2 years after the end of the study

---

Videotape Consent

I, the undersigned, hereby give my permission to be videotaped for the purposes described above.

Name: _____

Signature: _____

Relationship to study patient: _____

Date: _____

Signature/role of MISTIE III interviewer: _____

Date: _____

Appendix 5: Expected adverse event frequencies based on MISTIE II trial data.

Event	% Events (n=573)
Blood and lymphatic system disorders	**3.14%**
Anemia	2.27%
Leukocytosis	0.87%
Cardiac disorders	**5.89%**
Atrial fibrillation	0.87%
Atrial flutter	0.35%
Bradycardia	0.52%
Cardiac arrest	0.70%
Chest pain	0.35%
Congestive heart failure	0.17%
Dysrhythmia	0.17%
Myocardial infarction	0.52%
Prolonged QT interval on EKG	0.17%
Pulmonary vascular congestion	0.17%
PVC's; bigemeny	0.17%
Sinus bradycardia	0.17%
Sinus tachycardia	0.52%
Supraventricular tachydysrhythmia	0.52%
Ventricular extrasystoles	0.17%
Ventricular fibrillation	0.35%
Endocrine disorders	**0.17%**
Hypothyroid	0.17%
Eye disorders	**0.17%**
Vision abnormalities	0.17%
Gastrointestinal disorders	**5.89%**
Abdominal pain	0.35%
Colitis	0.17%
Constipation	1.22%
Diarrhea	0.87%
Dysphagia	0.70%
Esophagitis	0.17%
Gall bladder thickening	0.17%
Gastritis	0.17%
Gastrointestinal bleeding	0.70%
Ileus	0.17%
Increased gastric outputs	0.17%
Intraoperative hemorrhage (PEG)	0.17%
Peptic ulcer	0.17%
Rotten tooth extraction	0.17%
Small intestine infection	0.17%
Vomiting	0.35%
General disorders and administration site conditions	**6.44%**
Death due to index bleeding event	1.05%
Fever	4.19%
Generalized edema	0.17%
Localized edema	0.52%
Phlebitis	0.17%
Sudden death NOS	0.17%
Transient arm weakness	0.17%
Immune system disorders	**0.35%**
Anaphylaxis	0.17%
Angioedema	0.17%
Infections, non-neurologic	**7.13%**
Bacteremia	0.87%
Bronchial infection	0.17%
Clostridium difficile	0.35%
Endocarditis	0.17%
Enterocolitis infectious	0.35%
Pleural infection	0.17%
Sepsis	0.52%
Sinusitis	0.35%
Skin infection	0.17%
Upper respiratory infection	0.52%
Urinary tract infection	3.32%
Vaginal infection	0.17%
Investigations	**2.57%**
Coagulopathy	0.17%
Creatinine increased	0.17%
Eosinophilia	0.17%
Increased ALT	0.17%
Increased AST	0.17%
Increased bilirubin, direct	0.17%
Increased chloride	0.17%
increased d-dimer	0.35%
Increased fibrinogen	0.35%
Increased PT	0.17%
Patelet count decreased	0.17%
Thrombocytopenia	0.17%
Thrombocytosis	0.17%
Metabolism and nutrition disorders	**11.67%**
Acidosis (metabolic or respiratory)	0.35%
Dehydration	0.17%
Electrolyte imbalance	0.17%
Hyperglycemia	1.22%
Hyperkalemia	0.52%
Hypernatremia	0.70%

Hypertension	2.44%
Hypoalbuminemia	0.17%
Hypocalcemia	1.05%
Hypoglycemia	0.87%
Hypokalemia	1.40%
Hypomagnesemia	0.70%
Hyponatremia	0.87%
Hypophosphatemia	0.87%
Metabolic alkalosis	0.17%
Musculoskeletal and connective tissue disorders	**0.17%**
Back pain	0.17%
Nervous system disorders	**27.71%**
Anoxic brain injury	0.17%
Aphasia	0.35%
CSF leak	0.17%
Depressed level of consciousness	1.92%
Dizziness	0.17%
Edema cerebral	0.87%
Headache	1.57%
Herniation	0.17%
Hydrocephalus	0.35%
Intracranial hemorrhage: Catheter Tract, Enlargement	0.70%
Intracranial hemorrhage: Catheter Tract, New	4.01%
Intracranial hemorrhage: Hematoma, subdural	0.17%
Intracranial hemorrhage: Tissue, Enlargement	4.01%
Intracranial hemorrhage: Tissue, New	1.40%
Intracranial hemorrhage: Ventricular system, Enlargement	1.75%
Intracranial hemorrhage: Ventricular system, New	1.57%
Intracranial hypertension	1.75%
Ischemia cerebrovascular	1.92%
Mass effect	0.17%
Meningitis	0.17%
Muscle twitching	0.70%
Seizure	2.27%
Somnolence	0.17%
Stroke	0.17%
Syncope	0.70%
Ventriculitis, non-bacterial	0.17%
Wound reclosure after serous fluid leak	0.17%
Psychiatric disorders	**1.04%**
Agitation	0.87%
Labile sleep wake cycle	0.17%
Renal and urinary disorders	**2.61%**
Acute kidney injury	0.35%
Acute renal insufficiency	0.87%
Hematuria	0.35%
Left renal mass	0.17%
Urinary incontinence	0.17%
Urinary retention	0.70%
Respiratory, mediastinal and thoracic disorders	**19.84%**
Adult respiratory distress syndrome	1.22%
Aspiration	1.22%
Atelectasis	0.87%
Bronchospasm	0.17%
Dyspnea	0.17%
Epistaxis	0.87%
Hemothorax	0.17%
Hypoxemia	0.70%
Increased respiratory secretions	0.17%
Lung infection	1.22%
MRSA infection	0.17%
Pleural effusion	1.05%
Pneumonia	5.76%
Pneumothorax	0.17%
Pulmonary edema	0.70%
Respiratory arrest	0.17%
Respiratory failure	3.14%
Shortness of breath	0.35%
SIADH	0.17%
Stridor	0.17%
Tachypnia	0.52%
Tracheal stricture	0.17%
Tracheitis	0.35%
Wheezing	0.17%
Skin disorders	**0.35%**
Rash maculo-papular	0.35%
Vascular disorders	**4.02%**
Hypotension	1.05%
Thromboembolic event	2.97%

Appendix 6: Genentech Drug Safety: Safety Reporting FAX Cover Sheet

Genentech
A Member of the Roche Group

SAFETY REPORTING FAX COVER SHEET

Genentech Supported Research

AE / SAE FAX No: (650) 225-4682

Alternate Fax No: (650) 225-5288

Genentech Study Number	
Principal Investigator	
Site Name	
Reporter name	
Reporter Telephone #	
Reporter Fax #	

Initial Report Date	[DD] / [MON] / [YY]
Follow-up Report Date	[DD] / [MON] / [YY]

Subject Initials (Enter a dash if patient has no middle name)	[ ] - [ ] - [ ]

SAE or Safety Reporting questions, contact Genentech Safety: (888) 835-2555

PLEASE PLACE MEDWATCH REPORT or SAFETY REPORT BEHIND THIS COVER SHEET

Version 1 31-May-2012

Appendix 7: Abbreviation List

AAN:	American Association of Neurology	GOSE:	Glasgow Outcome Scale Extended
AANS:	American Academy of Neurological Surgery	GRE:	Gradient echo
ADL:	Activities of Daily Living	HIPAA:	Health Insurance Portability and Accountability Act
AE:	Adverse event	HOB:	Head of bead
AHA:	American Heart Association	hr, h, or hrs:	Hour(s)
AIC:	Akaike Information Criterion	HR:	Heart rate
aPTT:	Activated partial thromboplastic time	ICES:	Intraoperative CT guided Endoscopic Surgery for intracerebral hemorrhage trial
ASA:	Aspirin	ICH:	Intracerebral hemorrhage
ATACH:	Antihypertensive Treatment of Acute Cerebral Hemorrhage Trial	ICH:	International Conference on Harmonisation
AVM:	Arteriovenous malformation	ICP:	Intracranial pressure
BA:	Bioavailability	ICU:	Intensive care unit
BE:	Bioequivalence	IEC:	Independent Ethics Committee
BP:	Blood pressure	Inc:	Incorporated
CAPA:	Corrective and preventative action plan	IND:	Investigational new drug
cc:	Cubic centimeter	INR:	International normalized ratio
CCC:	Clinical Coordinating Center	INTERACT:	Intensive Blood Pressure Reduction in Acute Cerebral Haemorrhage Trial
CD:	Compact Disc	IRB:	Institutional Review Board
CES-D:	Center for Epidemiological Studies – Depression	IST:	Investigator sponsored trial
CFR:	Code of Federal Regulations	ITT:	Intention-to-treat
CHANT:	Cerebral Hemorrhage and NXY-059 Treatment	IV:	Intravenous
CI:	Confidence interval	IVH:	Intraventricular hemorrhage
CLEAR III:	Clot Lysis: Evaluating Accelerated Resolution of Intraventricular Hemorrhage: Phase III	JHMI:	Johns Hopkins Medical Institutions
cm:	Centimeter	JHU:	Johns Hopkins University
CRO:	Contract research organization	kcal:	Kilocalorie
CSF:	Cerebrospinal fluid	kg:	Kilogram
CT:	Computerized tomography	LOC:	Level of consciousness
CTA:	Computed tomography angiogram	LTF:	Lost to follow-up
CTCAE:	Common Terminology Criteria for Adverse Events	MAP:	Mean arterial pressure
CV:	Curriculum vitae	MD:	Doctor of Medicine
CVD:	Cardiovascular disease	med:	Medical
DBP:	Diastolic blood pressure	MedDRA:	Medical Dictionary for Regulatory Activities
DCC:	Data Coordinating Center	MEOI:	Medical event of interest
dCT:	Diagnostic Computed Tomography	mg:	Milligram
Diag:	Diagnostic	MHRA:	Medicines and Healthcare products Regulatory Agency
DICOM:	Digital imaging and communications in medicine	min:	minutes
DNI:	Do not intubate	MIS:	Minimally invasive surgery
DNR:	Do not resuscitate	MISTIE II:	MISTIE Phase II (precursor to MISTIE III)
DSMB:	Data Safety and Monitoring Board	MISTIE III:	Minimally Invasive Surgery plus rt-PA for ICH Evacuation Phase III
DVD:	Digital video disc	mL:	Milliliter
DVT:	Deep vein thrombosis	mmHg:	Millimeters of mercury
DWI:	Diffusion weighted imaging	MMSE:	Mini-Mental State Exam
EC:	Executive Committee	MOP:	Manual of Operations and Procedures
eCRF:	Electronic case report form	MR:	Magnetic resonance
ED:	Emergency Department	MRA:	Magnetic resonance angiogram
EDC:	Electronic data capture	MRI:	Magnetic resonance imaging
EKG:	Electrocardiogram	mRS:	Modified Rankin Scale
EOT:	End of treatment	MTI:M3	Mechanisms of Tissue Injury in MISTIE III (ancillary study)
EQ-5D:	Euroquol-5D	NIH:	National Institutes of Health
eTMF:	Electronic trial master file	NIHSS:	National Institutes of Health Stroke Scale
EUSI:	European Stroke Initiative	NINDS:	The National Institute of Neurological Disorders and Stroke
EVD:	Extraventricular drainage	OHRP:	Office of Human Research Protection
FAST:	Factor Seven for Acute Hemorrhagic Stroke Trial	OR:	Odds ratio
FDA:	Food and Drug Administration	OR:	Operating room
FFP:	Fresh frozen plasma	PACS:	Picture Archiving and Communication System
Fig:	Figure	PBSI:	Preference Based Stroke Index
G:	Gram	PHI:	Protected health information
GCP:	Good Clinical Practice	PI:	Principal investigator
GCP:	Good clinical practice	PT:	Prothrombin time
GCS:	Glasgow Coma Scale	Pts:	Patients
GEE:	Generalized estimating equations	Q:	Every
GLMM:	Generalized Linear Mixed Model	QA:	Quality assurance
gm:	Gram	QD:	Every day
GOS:	Glasgow Outcome Scale	QOL:	Quality of life
RDS:	Regulatory document specialist	RCT:	Randomized clinical trial
ROC:	Receiver Operating Characteristic	SIS:	Stroke Impact Scale
rt-PA:	Recombinant tissue plasminogen activator	SIS:	Stroke Impact Scale
SAE:	Serious adverse event	SOP:	Standard operating procedure
SAINT:	Stroke-Acute Ischemic NXY Treatment	SPRG:	Stroke Progress Review Group (NINDS Advisory and Peer Review Group)
SBP:	Systolic blood pressure	STICH:	Surgical Trial in Lobar Intracerebral Hemorrhage
SCDs:	Sequential compression devices	surg:	Surgical
SD:	Standard deviation	TIL:	Therapy intensity level
SDV:	Source document verification	t-PA:	Tissue plasminogen activator
SID:	Study identification number	VISTA:	Virtual International Stroke Trials Archive
		Vol:	Volume

Appendix 8: MTI-M3 Ancillary Study Protocol

Participation in the MTI-M3 ancillary study is optional. Study centers outside of the US and Canada will not participate in the portion of the protocol that requires overnight shipment of samples to Yale University. Otherwise, all study centers are eligible to participate but may opt-out.

Mechanisms of Tissue Injury in MISTIE III: Rebleeding and inflammation: Predicting risk of excessive bleeding in minimally invasive surgery and inflammatory marker evaluation.

1. Abstract

Intracerebral hemorrhage (ICH) is the most deadly and disabling form of stroke. One treatment option is surgical hematoma evacuation; however, two large trials of this intervention have failed to show benefit. The most promising current intervention is minimally invasive surgery (MIS), the subject of the MISTIE III clinical trial. This ancillary protocol, Mechanisms of Tissue Injury in MISTIE III: Rebleeding and Inflammation (MTI:M3) will be an ancillary study to the MISTIE III trial. The overriding goals of this ancillary study are to determine which patients are most likely and least likely to benefit from MIS and whether the level of activation of central nervous system inflammatory mediators is associated with ICH severity, perihematomal edema, and recovery.

2. Study Objectives

MTI:M3 will examine neuroimaging together with blood and intrahematomal fluid biomarkers in patients enrolled in MISTIE III. The objectives are as follows: (1) First, we will determine whether the presence of the CTA spot sign, a marker of bleeding risk, can mark those patients at highest risk of perioperative bleeding and lowest likelihood of benefit following MIS. (2) We will determine whether markers of small vessel disease, including cerebral microbleeds (CMB) and white matter hyperintensities, are associated with perioperative complications and opportunity to benefit from MIS. (3) We will determine whether intrahematomal and peripheral markers of inflammation and the cellular immune response are associated with hematoma and intraventricular clot volume, perihematomal edema and outcomes; (4) We will determine whether genetic markers are associated with perioperative complications, the central inflammatory response and outcome following MIS. The results of the proposed study will have direct impact on both clinical care and research. If MISTIE III shows improved outcome after ICH, our results will provide neurosurgeons with the data to determine which patients are the most optimal candidates for the procedure. If MISTIE III does not show a benefit for the overall cohort, our results may still highlight that certain patient subgroups can in fact benefit, and that those patients can be identified in the acute phase. The investigation of intracranial inflammatory cascades in MISTIE III represents a unique opportunity to understand immune responses and potentially identify new targets to limit inflammation and improve outcomes following ICH. Simultaneous analysis of peripheral and central inflammatory responses will for the first time provide direct clinical correlation between the brain and serum inflammatory responses in spontaneous ICH. If an association does exist, then blood surrogates could help gauge the degree of central inflammation.

Overall, MTI:M3 offers a tremendous opportunity to leverage clinical trial data to bring novel insights from ICH pathophysiology into the clinical realm, using neuroimaging, genetic and

inflammatory markers of disease to provide clinicians with powerful new tools to guide surgical therapy and develop new therapeutic targets.

- **Specific Aim 1**. To determine whether CTA spot sign is associated with perioperative bleeding and hematoma reduction after MIS

- **Specific Aim 2**. To determine whether white matter hyperintensity burden and cerebral microbleeds are associated with perioperative bleeding and outcome after MIS.

- **Specific Aim 3a**. To identify the association between acute phase levels of intracranial and peripheral inflammatory mediators and hematoma volume, and perihematomal edema as determined by cerebral imaging.

- **Specific Aim 3b**. To determine whether acute phase levels of intracranial inflammatory markers are associated with 90/180 day functional outcomes in intracerebral hemorrhage.

- **Specific Aim 4a**. To determine if specific genotypes are associated with perihematomal edema and outcome after MIS.

- **Specific Aim 5a**. To determine the cellular immune responses to acute brain hemorrhage and how these responses change over time.

- **Specific Aim 5b**. To determine whether cellular immune responses transition from pro-inflammatory to those that aid in resolution of inflammation are associated with ICH clearance and improved functional recovery.

3. Background

Intracerebral hemorrhage (ICH) hematoma size is a potent predictor of poor outcome in ICH. Minimally invasive surgery (MIS) to aspirate and reduce hematoma volume is a promising therapy that may reduce neurotoxicity and brain injury. MISTIE III is a randomized controlled trial examining the relationship between outcome following ICH and MIS. It builds on MISTIE II, which found that one limitation to MIS was variable success in hematoma volume reduction. In some patients, portions of the clot could not be aspirated, or blood re-accumulated, or secondary effects from clot formation may be greater, mitigating the benefit of this procedure. While catheter placement plays a fundamental role, accumulating evidence suggests that patient-specific factors influence the extent of bleeding in ICH and the therapeutic response to ICH surgery.

Our team has demonstrated that contrast extravasation on CT angiography (CTA spot sign) identifies ICH patients at highest risk for hematoma expansion and ongoing bleeding. Furthermore, among patients undergoing surgical hematoma evacuation, CTA spot sign strongly predicted peri-operative and post-operative re-bleeding. Additional genetic and imaging data support the hypothesis that the type and severity of the underlying cerebral small vessel disease associated with ICH will influence outcomes, side effects, and the extent of bleeding. Our team has also demonstrated that in cerebrospinal fluid of patients with spontaneous intraventricular hemorrhage (IVH) treated with external ventricular drainage, maximally elevated mean levels of interleukins, IL-1beta, IL-10 and IL-8 occur on post bleed day

1, which subsequently plateaued around post bleed day 5, before returning to normal by day 10. Levels of monocyte chemo-attractant protein (MCP-1) showed persistent elevation over 2 weeks. The time trends of these markers support experimental data that suggests inflammation is a contributor to acute brain injury after ICH/IVH.

4. Study Procedures

MTI:M3 is a prospective and observational, nested study within the MISTIE III randomized controlled clinical trial. This design provides tremendous leverage of the existing workload for MISTIE III, promising major insight into neuroimaging and genetic and inflammatory biomarkers of ICH pathophysiology and response to surgical intervention without affecting the execution of the parent study.

The primary goal of MTI:M3 is to determine whether radiologic and genetic and inflammatory marker data can help identify those ICH patients most likely to undergo successful clot reduction after the MIS intervention and most likely to derive clinical benefit from the procedure. Details of MISTIE III are not reprinted here. Incorporation of MTI:M3 will require an additional informed consent section in the MISTIE III consent for lab draw and collection of hematoma fluid from the collection bag/chamber. All patients enrolled in MISTIE will be eligible for MTI:M3.

a. The study will be completed as part of the participants' hospitalization and follow-up.

b. Imaging required for the MTI:M3 study will be done as part of the MISTIE III protocol. Image files will be uploaded to the MISTIE III coordinating center via the electronic data capture system. Image files collected from subjects who also consent to MTI:M3 will then be de-identified and electronically transferred to MTI:M3 investigators for analysis as described below in section 6.

c. There are 2 types of blood draws:

    (i) A daily blood draw (two teaspoons, 12 mL) on the day of enrollment into MISTIE III (Day 0) (for both medical and surgical patients) and daily for up to 4 days timed with routine collection of blood for daily blood tests (medical patients), or with hematoma fluid aspiration (surgical patients). Samples will be collected in two separate tubes, one shipped immediately and the other locally processed (centrifuged and aliquoted), frozen at -70°C, and shipped to specific investigators for storage and analysis.

    (ii) A separate single blood draw (approximately 1 teaspoon, one 6 mL tube) will be collected from medical and surgical MISTIE III subjects at any time during the hospitalization or during follow-up. The 6mL tube of blood will be locally processed (centrifuged and the plasma aliquoted into cryovials) and frozen at -70°C. <u>For sites not able to participate in part (d) below:</u> during this separate blood draw, two additional tubes (10 mL each, approximately 4 teaspoons total) of whole blood will also be collected and frozen at -70°C. The frozen whole blood and plasma from this single draw will be shipped to specific investigators for storage and analysis.

d. A 1-10 cc sample of the patient's hematoma drainage from the existing brain catheter will be collected on the following schedule:

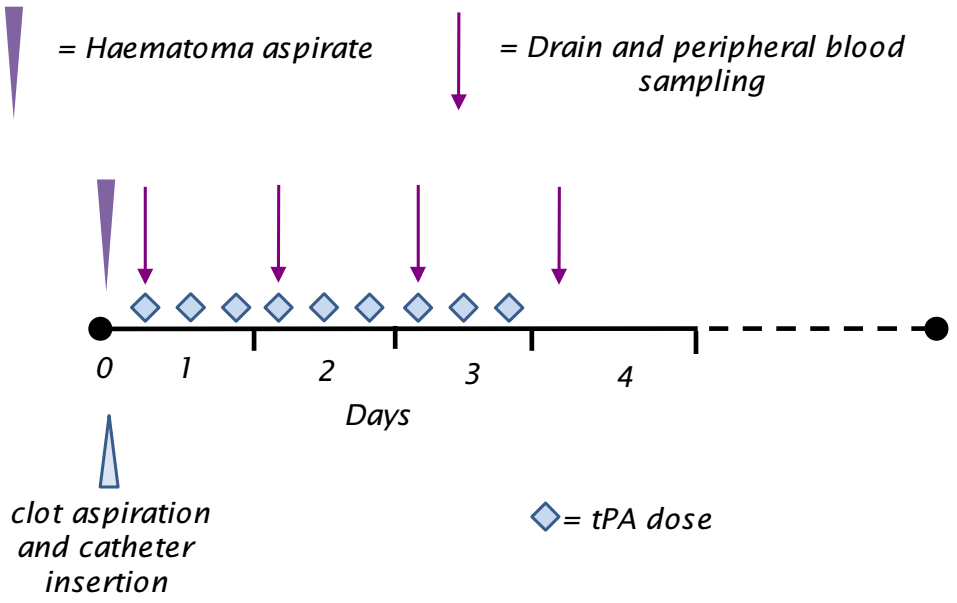
(i) At time of initial placement of the brain catheter (at clot aspiration and catheter insertion= "Hematoma aspirate").

(ii) Once daily from the drainage bag/collection chamber to be timed immediately prior to injection of rtPA through the brain catheter (= "Drain sampling").

(iii) From the drainage bag/collection chamber at the time of removal of the brain catheter.

(iv) Samples will divided and collected in two separate tubes, one shipped immediately and the other locally processed (centrifuged and aliquoted), frozen at -70°C, and shipped to the specific investigators for storage and analysis.

Procedures for Perihematomal Fluid Sampling in MISTIE



e. Additional data collected as part of the MISTIE III protocol will also be made available to MTI:M3 investigators. These data include but are not limited to: study subject ID number, demographic and baseline variables including recent infection

and underlying inflammatory conditions, MIS procedure, rt-PA dosing, outcomes scales scores (blinded data; available after database lock), and adverse events.

5. Inclusion/Exclusion Criteria

The participant must be an adult (older than 18 years of age) enrolled in the parent trial for this proposal, MISTIE III.

6. Drugs/Substances/Devices

None.

7. Study Statistics

- For Aim 1a, to evaluate whether Spot Sign absence predicts clot reduction after MIS, we will analyze clot reduction in two ways: first as a continuous variable compared using a two-sample t-test, then as a dichotomized variable (<60% vs. ≥60%, with ≥60% defined as successful clot reduction) compared using a chi-square test.

- For Aim 1b, we will evaluate whether Spot Sign is associated with increased frequency of neurological complications.

- For Aim 2a, we will evaluate whether white matter disease burden is associated with neurologic complications after MIS.

- For Aim 2b, we will evaluate whether patients with CMBs have less frequent neurological complications.

- For Aim 3a, we will compare serially measured inflammatory markers from serum, and hematoma drainage with ICH, IVH and PHE volumes using standard statistical techniques.

- For Aim 3b, we will assess the association between mean levels of inflammatory mediators and 90/180-day mRS.

- For Aim 4, we will evaluate whether genotype predicts neurological complications after MIS.

- For Aim 5, we will determine the infiltration of peripheral leukocytes into the hematoma and the transcriptional profile of multiple leukocyte subsets in order to determine how leukocyte responses correlate with outcome.

Data analysis for Aim 1 (Clot reduction and rebleeding). To evaluate whether Spot Sign absence predicts clot reduction after MIS. Clot reduction will be analyzed in two ways: first as a continuous variable compared using a two-sample t-test, then as a dichotomized variable (<60% vs. ≥60%, with ≥60% defined as a successful clot reduction) compared using a chi-square test. This analysis will focus on the surgical management arm. Based upon prior work, we expect 34% of patients to show a Spot Sign. With a conservative estimate of 30% for the

SD of clot reduction and a sample size of 85 and 165 for Spot Sign positive and negative, respectively, the study will have 80% power to detect a mean difference of 11.3% in clot size reduction. When dichotomized at 60%, the study will have 82% power to detect a difference of 20% (40% for the spot sign positive and 60% for the spot sign negative) with a 0.05 two-sided significance level.

Secondary analyses will include outcomes of severe rebleeding with neurologic deterioration (expected to occur 3.6% of the time), rebleeding into the surgical bed as a radiographic outcome (expected to occur 15% of the time), and bleeding along the MIS catheter tract (expected to occur 30% of the time).

Data analysis for Aim 1 (Clinical outcomes). To evaluate whether Spot Sign is associated with improved clinical outcome associated with MISTIE III study intervention.

The primary outcome in MISTIE III is good functional outcome, defined by modified Rankin Score (mRS) of 0-3 at 180 days following treatment. We hypothesize that treatment effect will be more profound among patients who are absent of a Spot Sign. This will be tested using Wald's test for the interaction effect between treatment and Spot Sign in a generalized linear model. The MISTIE III study has 88% power to detect a 13% difference in the dichotomized outcome between the control and intervention group. Our assumptions on the proportion with mRS 0-3 for the four groups are shown in the table below.

	Medial management arm	Surgical management arm
Spot Sign present	18% (N=85)	18% (N=85)
Spot Sign absent	27% (N=165)	44% (N=165)
Total	24% (N=250)	35% (N=250)

With a simulated sample, we found that the study has only 43% power for testing the interaction between treatment and spot sign. However, among the 330 patients without spot sign, the study will have 87% power to detect the 17% difference between treatment arms with a two sided 0.05 significance level.

Specific Aim 2 (White matter disease burden). To determine the association between white matter disease burden with successful clot reduction after MIS.

Local MISTIE III sites will electronically transfer MRI images to the MISTIE III coordinating site via the electronic data collection system, which will then be electronically transferred to the MTI:M3 investigators. Specific scanner type and GRE imaging parameters will be documented.

Volumetric analysis of white matter disease will be performed. This analysis will focus on the surgical management arm and white matter disease burden will be compared between those with and without successful clot reduction (≥60%) using a two-sample t-test. Assuming 53% of

the patients will achieve successful clot reduction and a SD of 6.9cc, the study will have 80% power to detect a mean difference as small as 2.5cc with a 0.05 two-sided significance level.

Specific Aim 2 (Microbleeds). To determine whether patients with microbleeds have a higher likelihood of successful clot reduction after MIS.

Identification of CMBs and determination of their number, size, and distribution will be performed by two trained reviewers blinded to clinical and radiographic information. CMBs will be identified according to criteria proposed by the Microbleed Study Group and in long-term use at the MTI:M3 coordinating center. Briefly, these criteria require that identified CMB be black or substantially hypointense on T2*-weighted MRI, round or ovoid (excluding tubular or linear structures that may represent flow voids or resorbed macrobleeds), blooming (i.e. larger on T2*-weighted than spin-echo MRI), devoid of T1- or T2-weighted hyperintensity, and at least half surrounded by brain parenchyma (to exclude primarily subarachnoid bleeding). Our group has previously reported very high inter-rater reliability for detection of CMB using either conventional T2*-weighted gradient-echo MRI (intra-class correlation coefficient =0.97) or the more sensitive susceptibility-weighted imaging technique (SWI; intra-class correlation coefficient =0.93). Other parameters to be measured on MRI will include the presence of perihematomal edema, extravasation of MRI contrast, and presence of diffusion/perfusion abnormalities. Volumetric analysis of white matter hyperintensity will be performed as previously described, as will evaluation for CAA using the Boston criteria (originally developed by our group).

The primary hypothesis is that presence of CMBs on GRE-MRI is associated with more successful clot reduction. This analysis will focus on the surgical management arm. We predict there will be an association between absence of CMBs, and proportional change in hematoma volume. Similar to Aim 1a above, clot reduction will be analyzed as a continuous variable compared using a two-sample t-test, and as a dichotomized variable compared using a chi-square test. We expect 31% of patients to show signs of CMBs. Assuming a SD of 30% and a sample size of 77 and 173 for any CMBs and no CMBs, respectively, the study will have 80% power to detect a mean difference of 11.6% in clot size reduction. When dichotomized at 60%, the study will have 80% power to detect a difference of 20% (67% for the any CMBs group and 47% for the no CMBs group) with a 0.05 two-sided significance level.

Finally, we propose to evaluate whether the difference in neurologic outcome between the MISTIE III intervention and control is greatest in those with CMBs on GRE-MRI. Secondary analyses will examine the interaction between CMBs, Spot Sign, white matter burden, and neurologic outcome. The model for clinical benefit (i.e. mRS 0-3 vs. 4-6) will include effects for treatment, CMBs, and the interaction between treatment and CMBs. Wald's test will be used to assess the significance of the interaction effect. These results can be confirmed using the CMH test.

Specific Aim 3a/b. To identify the association between acute phase levels of intracranial and peripheral inflammatory mediators and hematoma volume, peri-hematomal edema and neurologic outcomes as determined by cerebral imaging and mRS.

The primary outcome measure of this aim is serial levels of inflammatory mediators in hematoma fluid/surgical aspirate. The secondary outcome measures are: (i) ICH/IVH clot burden on admission and end of treatment and absolute/relative perihematomal edema (PHE) at admission and end of treatment (24 hrs post brain catheter removal) compared to time of brain catheter placement; (ii) functional outcomes measured by mRS at 90 and 180 days after admission; and (iii) serial levels of inflammatory mediators in serum. Hematoma aspirate and serum inflammatory markers (see Table below) will be determined by the following methods:

White blood cell count and differential, CRP, glucose, protein, red blood cell count, fibrinogen, and fibrin D-dimer (DD) will be measured using routine laboratory assays.

Concentrations of IL-1α, IL-2, IL-6, TNFα, IL-8, IL-10, (MCP)-1, (MIP)-1α,IL-1 receptor antagonist, IL-10, TGF-β and IFNγ will be quantified using immunoassays.

Levels of matrix metalloproteinases (MMPs) will be assayed by two-step sandwich enzyme-linked immunosorbent assay methods.

Glutamate concentrations will be analyzed by high-performance liquid chromatography.

Inflammatory Marker Studies	
Serum Studies	D-dimer, C-reactive protein (CRP), fibrinogen, matrix metalloproteinases (MMPs), Interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), glutamate
Hematoma Aspirate Studies	White blood cell count and differential, glucose, protein, red blood cell count, Interleukin-1 (IL-1), Interleukin-2 (IL-2), Interleukin-6 (IL-6), Interleukin-8 (IL-8), IL-10, monocyte chemoattractant protein(MCP)-1, macrophage inflammatory protein (MIP)-1α, tumor necrosis factor-α (TNFα), Interferon-gamma (IFNγ), transforming growth factor(TGF)-β, matrix metalloproteinases (MMPs)

Statistical plan: Analysis of categorical variables will be performed using the Pearson's Chi-square test. The Fisher exact test will be used when applicable. Continuous variables will be analyzed using one-way Analysis of variance if data is normally distributed or with Kruskall-Wallis test if not normally distributed. Trend statistic of inflammatory markers will be calculated using the Mantel-Haenszel test for linear association, with the catheter day plotted on the X-axis and levels of specific inflammatory mediators on the Y-axis.

For analysis of secondary outcome measures, inflammatory markers will be expressed as AUC (area under the curve) and included in a multifactorial linear regression model to test for associations with ICH/IVH/PHE volumes and with 90/180-day mRS. The models will include age, sex, and ICH score as covariates. Given the anticipated small sample size of the study population, a total of four or fewer confounders can be controlled in regression models. All tests will be two-tailed, with significance defined by $p<0.05$. Statistical analysis will be performed using STATA 12.0 (Stata, College Station, Texas).

Specific Aim 4a. ApoE Genotype
Secondary analyses will explore whether imaging and genetics are associated with specific subcategories of neurological complications, whether they predict improved neurological outcome, and whether they stratify who will receive the most clinical benefit from the MIS intervention

We will test the hypothesis that specific ApoE genotype predicts successful clot reduction after MIS. This analysis will focus on the surgical management arm. We predict those patients with an E2 allele will have less clot reduction. Similar to Aim 1a, clot reduction will be analyzed as a continuous variable compared using a two-sample t-test, and as a dichotomized variable compared using a chi-square test. We expect 9% of patients will have an ApoE E2 allele. Assuming a SD of 30% and a sample size of 22 and 228 for presence of an ApoE E2 allele vs. absence, respectively, the study will have 80% power to detect a mean difference of 18.8% in clot size reduction. When dichotomized at 60%, the study will have 84% power to detect a difference of 24% (22% for the ApoE E2 allele group and 56% for the no ApoE E2 allele group) with a 0.05 two-sided significance level.

Specific Aim 4b. Haptoglobin phenotype
We will test the hypothesis that haptoglobin 1-1 predicts better outcome. For our analysis of haptoglobin, patients will be categorized as Hp1-1, 1-2, or 2-2 phenotype. Our primary analysis will examine whether Hp1-1 predicts a higher likelihood of good neurologic outcome (defined as above) using a chi-square test. Our preliminary data suggests that that 13% of patients will have the Hp1-1 phenotype, 48% will have Hp 1-2, and 38% will have 2-2. We do not anticipate interaction between haptoglobin genotype and treatment effect; therefore, the analysis will be conducted combining the two treatment arms. Assuming a sample size of 65 and 435 for Hp1-1 and Hp (1-2 + 2-2), respectively, the study will have 82% power to detect a difference of 19% (46% for the Hp 1-1 group and 27% for the other 2 genotypes) with a 0.05 two-sided significance level.

Specific Aim 5. Leukocyte infiltration and outcomes. Our preliminary data from both mice and human patients demonstrates a robust recruitment of peripheral leukocytes into the hematoma and perihematomal region over the first seven days after ICH. During this time, blood derived macrophages transition from a pro-inflammatory phenotype to a reparative phenotype that aids in phagocytosis of the hemorrhage and wound healing. Cell surface markers on macrophages involved in phagocytosis, including CD36, and inhibition of T cell response, including PDL2, increase over this time. Our primary hypothesis is that this transition to repair is critical to good outcome after ICH, and that patients who transition to reparative macrophage profiles and shifts from effector to regulatory T cell responses earlier after ICH onset will have improved outcomes.

Hematoma aspirate and blood samples will be shipped ambient priority overnight to Yale University and immediately analyzed to prevent degradation of samples. Mononuclear cells will be isolated by Ficoll gradient, stained with fluorophore-conjugated antibodies to CD45, CD11b, CD16, CD3, CD4, CD8, CD25, and CD127. Leukocytes will be quantified by flow cytometry and cell sorting (FACS) to isolate populations of interest (see Table below for the gating of the populations to be sorted). In a single batch, RNA from each population of interest will be converted to cDNA and analyzed by RNAseq to fully characterize the transcriptional profile of

each cell population at each time point after ICH onset. Peripheral blood samples will be processed and analyzed in the identical fashion to allow for direct comparison of the differences in cellular phenotypes that occur once the cells have migrated into the brain. Analyses will compare (1) blood to brain transcriptional profile differences at each time point, (2) changes in transcriptional profiles within leukocyte populations over time, (3) initial transcriptional profiles of macrophage population and ICH severity, (4) transcriptional profile shifts to reparative/suppressor macrophage and T cell phenotypes and outcome at 90/180 days. RNA sequencing and analysis will be performed by collaborator J. Christopher Love, PhD, at the Broad Institute of MIT and Harvard.

Cell surface markers	Leukocyte population	Analysis plan
$CD45^{hi}CD3^{-}CD11b^{+}CD16^{+}$	Blood derived macrophage	Quantified, sorted, and analyzed by RNAseq
$CD45^{hi}CD3^{+}CD4^{+}CD25^{-}CD127^{hi}$	Effector TH1 lymphocytes	Quantified, sorted, and analyzed by RNAseq
$CD45^{hi}CD3^{+}CD4^{+}CD25^{+}CD127^{-}$	Regulatory T cells	Quantified, sorted, and analyzed by RNAseq
$CD45^{hi}CD3^{+}CD4^{-}CD8^{+}$	Cytotoxic T cells	Quantified, sorted, and analyzed by RNAseq
$CD45^{lo}CD11b^{+}$	Microglia	Quantified by flow cytometry (brain only)
$CD45^{hi}CD3^{-}CD11b^{+}CD14^{hi}$	Blood classical monocyte	Quantified by flow cytometry (blood only)

8. Risks

Blood draws: There are no known medical risks to the patient except those associated with a blood draw from an indwelling line or a peripheral blood draw (both medical and surgical arms). We have minimized the number of blood draws so that they are timed with routine blood draws or with hematoma fluid aspiration and this will occur through an existing in-dwelling line or peripheral blood draw.

Drainage collection: In the surgical arm, the hematoma drainage in patients with a brain catheter will initially be obtained as part of its placement in the operating room during which the blood clot is aspirated as part of the MISTIE III parent protocol. This poses no additional risk or exposure of the volunteer to additional procedures. The subsequent samples will be collected daily from the spontaneous hematoma drainage into the collection bag/chamber using sterile technique. The only risk is infection, which is minimal for both hematoma fluid and peripheral blood collection.

No additional CT scans are required.

There are no legal ramifications to the participant.

There are no financial risks to the participant.

All unanticipated problems or study deviations will be reported to the PI who will be responsible for reporting them to the IRB.

9. Benefits

This may result in improved treatment of ICH for society in general. The participant will have the satisfaction of helping others through their participation.

10. Payment and Remuneration

There are no forms of compensation to the participant. The participants' care and ability to participate in the MISTIE III trial will not be impacted if they decide not to participate in this ancillary study.

11. Costs

There are no costs to the patients. Any costs associated with the blood draw will be paid by the investigator. The imaging will be done as part of the MISTIE III protocol.