INSIGHT: **In**ve**s**ti**g**ation of Clot in Ischemic Stroke and **H**ematoma Evacua**t**ion

Protocol
CLP-15131.E

Date of Protocol
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Sponsor
Penumbra, Inc.
One Penumbra Place
Alameda, CA 94502
USA

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Registry Title:	**INSIGHT: Investigation of Clot in Ischemic Stroke and Hematoma Evacuation**
Primary Objectives:	The aim of the study is to collect and analyze specimen from ischemic stroke patients undergoing thrombectomy procedures and from patients undergoing minimally invasive surgery for intracranial hematoma evacuation. Clot and blood specimen will be analyzed via histology, RNA sequencing, Single Nucleotide Polymorphisms (SNPs), and proteomic analysis.
Secondary Objective:	To prospectively collect and correlate comprehensive data relating patient's clinical presentation, hospital course, and length of hospital stay to specimen histology, RNA sequencing, SNPs, and proteomic analysis.
Registry Study Design:	This will be a prospective, multi-center, registry for the collection and analysis of specimen collected from ischemic stroke thrombectomy procedures and minimally invasive interventions for intracranial hematoma evacuation utilizing the Penumbra Aspiration Pump.
Indication:	Per FDA 510(k) cleared Indication stated in the Instructions For Use. (IFU)
Registry Device:	Penumbra Aspiration Pump
Patient Population:	Up to 400 patients will be enrolled from up to 30 sites in the United States. (US) Specimen will be analyzed via histology, RNA sequencing, SNPs, and proteomics.
Study Duration:	It is anticipated that enrollment will take approximately 2 years. Each patient may be in the registry for approximately 90 days from enrollment to last optional follow-up.
Follow-up:	Follow-up assessments will occur at discharge and an optional phone visit at approximately 90 days following the procedure.
Inclusion Criteria:	1. ≥ 18 Years of Age 2. Frontline treatment with: • Penumbra System for ischemic stroke patients eligible for mechanical thrombectomy or • Artemis Neuro Evacuation device for intracranial hematoma evacuation in patients eligible for minimally invasive surgery (MIS) 3. Extracted thrombus/embolus 4. Informed consent is obtained from either patient or legally authorized representative (LAR)
Exclusion Criteria:	1. Pregnancy or positive pregnancy test according to site routine practice (only required for women of childbearing potential; serum or urine acceptable) 2. Currently participating in an investigational drug or device clinical trial that may confound the ability to capture clot and/or influence clot composition.

CLP-15131 Protocol Synopsis	
Registry Title:	**INSIGHT: Investigation of Clot in Ischemic Stroke and Hematoma Evacuation**
	Patients in observational, natural history, and/or epidemiological studies not involving intervention are eligible.
Statistical Methods:	No formal statistical testing is planned for this study. All analyses will be descriptive and considered as hypothesis generating for future studies.

1. Introduction

Globally, cerebrovascular accidents are a leading cause of death and disability.[1] In 2013, an estimated 10.3 million new strokes were identified, of which two thirds were ischemic (incidence 99-132 per 100,000) and one third were hemorrhagic (incidence 56-65 per 100,000) or other non-ischemic cases.[2]

The recent publication of multiple randomized controlled trials established the efficacy of mechanical thrombectomy in treating stroke and have dramatically changed first-line treatments and recommendations for acute ischemic stroke.[3, 4] Clinical studies have also addressed hemorrhagic stroke, for which current surgical options and therapies are more limited[5]; treatments for this stroke subtype focus on the evacuation of hematoma and removal of clot volume using minimally invasive techniques.[6-9]

Despite these rapid advances in stroke treatment, mortality remains high: ischemic stokes accounted for 3.3 million deaths in 2013, and hemorrhagic strokes for an additional 3.2 million deaths globally.[2] Rates of global mortality in 2013 were 57.3 per 100,000 people for ischemic stroke and 52.8 per 100,000 people for hemorrhagic stroke.[10]

In ischemic stroke, good clinical outcomes are often contingent on timely clot removal to attain revascularization.[3, 11] Consequently, understanding clot characteristics could inform vital pre-treatment decisions and guide secondary prevention strategies by revealing stroke etiology.[12] Historically, data from stroke-implicated clot has been scarce, but the expansion of contemporary endovascular technologies provides an ideal opportunity to collect thrombus material for direct study.[12]

1.1 Thrombus Composition

Clots removed during endovascular procedures can differ in consistency and removability[13], leading to an increased focus on thrombus composition as a crucial variable for improving outcomes in both AIS and minimally invasive hematoma evacuation patients. Clot age[14], component ratios[15], and etiology[16-18] can each affect the structural properties of a clot, impacting success rates of thrombolytic treatments[15, 19] and the choice of clinical intervention[18].

For example, thrombus maturation results in decreased mechanical compliance due to progressive fibrin cross-linking and collagen deposition.[14] These lysis-resistant fibrin clots can be more recalcitrant to endovascular treatment and are predictive of adverse patient outcomes.[20] By contrast, erythrocyte-rich thrombi are associated with improved efficacy of endovascular thrombectomy.[21-23] A recent study of patients undergoing intra-arterial thrombectomy with either a stent or suction device showed that clots retrieved from successful recanalization trials had a higher proportion of red blood cells (RBC) than those from non-recanalization trials; these RBC-rich clots were also associated with the presence of a hyperdense clot sign during initial brain imaging.[23]

Thrombus composition can also depend on patient predisposing factors, including Diabetes mellitus, cardiovascular disease, statin use, and anticoagulant use (e.g. vitamin-K antagonists, non-vitamin K oral anticoagulants, antiplatelet agents).[24-26] These conditions affect clot structure and fibrin content, indicating that patient history may become an important predictor of targeted and successful treatment strategies.

1.2 Conclusion and Rationale

This prospective study will be conducted on ischemic stroke and intracranial hematoma evacuation cases. Clot removed during endovascular therapy and minimally invasive hematoma evacuations will be collected for histological, RNA sequencing, SNPs, and proteomic analysis.

More specifically, this study will examine whether clot composition, genetic, and proteomic variables can provide insight into thrombus etiology and/or have impact on clinical outcomes in patients with outwardly similar initial presentations.

This registry is not intended to be utilized or support a labelling/indication change in the United States.

2. Risk Analysis

This study uses FDA 510(k) cleared medical devices per indication in the cleared labeling. The overall risk profile for the Penumbra Aspiration Pump is low, as the devices do not come into contact with the patient. Risk analysis was performed as part of design control requirements of the Quality System Regulation (21 CFR 820).

There are risks associated with the clot evacuation procedure and should be discussed with potential patients according to each institution's routine practice.

2.1 Risk to Patients

The primary risk in this study is a potential breach of confidentiality. To minimize the risk, all study records containing protected health information (PHI) will be de-identified and assigned a unique patient identification number. Protocols will be in place for the handling and destruction of collected specimen.

Blood collection has minimal risk and will be performed during the procedure through the existing procedural vascular access per institution routine practice.

3. Registry Overview

3.1 Study Design

This is a prospective, multicenter, registry for the collection and analysis of specimen collected from ischemic stroke thrombectomy procedures and minimally

invasive interventions for intracranial hematoma evacuation procedures utilizing the Penumbra Aspiration Pump.

Patients eligible for thrombectomy or minimally invasive surgery, and who meet the inclusion and none of the exclusion criteria will be considered for enrollment. Treatment will be per site routine practice, using FDA cleared devices.

Patients will be considered enrolled upon signed informed consent and specimen collection. Specimen collected during the procedure will be shipped to a study Core Lab(s) for analysis.

Data for each patient will be collected at the time of enrollment and at each subsequent follow-up visit. Please refer to the Schedule of Assessments (Table 1) below for details.

3.2 Study Objectives

3.2.1 Primary Objectives

- The aim of the study is to collect and analyze specimen from ischemic stroke patients undergoing thrombectomy procedures and from patients undergoing minimally invasive surgery for intracranial hematoma evacuation. Clot and blood specimen will be analyzed via histology, RNA sequencing, Single Nucleotide Polymorphisms (SNPs), and proteomic analysis.

3.2.2 Secondary Objectives

- To prospectively collect and correlate data relating patient' s clinical presentation, hospital course, and length of hospital stay to specimen histology, RNA sequencing, SNPs, and proteomic analysis.

4. Study Population

Up to 400 patients will be enrolled from up to 30 sites in the United States. Specimen will be analyzed via histology, RNA sequencing, SNPs, and proteomics.

4.1 Inclusion Criteria

1. ≥ 18 Years of Age
2. Frontline treatment with:
   - Penumbra System for ischemic stroke patients eligible for mechanical thrombectomy or
   - Artemis Neuro Evacuation device for intracranial hematoma evacuation in patients eligible for minimally invasive surgery (MIS)
3. Extracted thrombus/embolus

4. Informed consent is obtained from either patient or legally authorized representative (LAR)

4.2 Exclusion Criteria

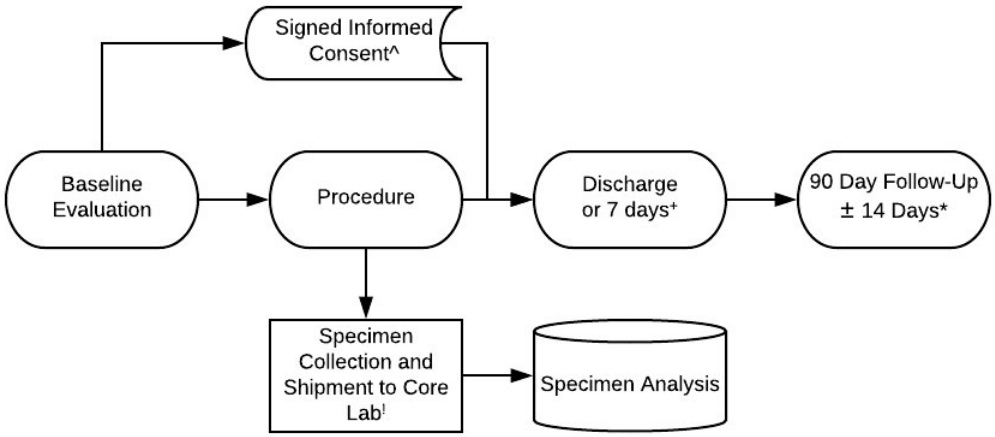
1. Pregnancy or positive pregnancy test according to site routine practice (only required for women of childbearing potential; serum or urine acceptable)
2. Currently participating in an investigational drug or device clinical trial that may confound the ability to capture clot and/or influence clot composition. Patients in observational, natural history, and/or epidemiological studies not involving intervention are eligible.

5. Study Procedures

5.1 Overview of Study Flow

Screening information will be reported in the Screening and Enrollment Log.

Figure 1: Study Flow



^ May be obtained up to 1 calendar day post-procedure, prior to specimen shipment to Core Lab
! Must be shipped as quickly as possible, but no later than 72 hours post procedure
+ Whichever comes first
* Optional Visit (conducted by phone)

5.2 Study Visits

Patients enrolled in this study will follow the visit schedule below. Procedure is considered day 0 to determine subsequent follow-up visit dates.

- Baseline Evaluation
- Procedure
- Discharge or 7 Days (whichever occurs first)
- 90 Day Follow-Up ± 14 Days (Optional phone call)

5.3 Screening and Baseline Evaluation

Patients will be clinically evaluated per site routine practice. The following information will be collected at Baseline:

- Medical History
- Demographics
- Clot/Hematoma Location
- Medications (including antiplatelet and anticoagulant use)
- The National Institutes of Health Stroke Scale (NIHSS)
- Glasgow Coma Score (GCS) – (MIS only)
- Historical (Pre-stroke) Modified Rankin Score (mRS)

5.4 Informed Consent

The Investigator or designee will obtain written informed consent from the patient or approved delegate using the current IRB approved consent form per IRB policy.

The patient or legally authorized representative (LAR) may provide informed consent up to one calendar days post-procedure.

All informed consent documents used under this protocol will be consistent with applicable elements of I.S. EN ISO 14155, Clinical investigation of medical devices for human subjects – Good Clinical Practice and 21 CFR Part 50 and will be approved by the site's reviewing IRB prior to registry initiation.

- Any modification to the sample informed consent form made by the study site must be approved by the Sponsor and the IRB before use. Each study site will provide the Sponsor with a copy of the IRB approved consent forms.
- Informed consent completion will be monitored regularly by the Sponsor.

5.5 Procedure

All procedures are to be conducted per routine practice at each study site.

The following information may be captured in the Case Report Form (CRF):

- Administration of intravenous thrombolytics (IV rtPA), Anti-coagulation agents, and medications
- Imaging times and results
  - Modified Thrombolysis in Cerebral Infarction (mTICI) scores pre and post procedure (AIS only)
  - Pre and post procedure hematoma volume (MIS only)
- Key procedural time points
- First pass to specimen extraction (AIS only)
- SAE collection

5.5.3 Specimen for Acute Ischemic Stroke Cases

Specimen will be collected during the procedure in the filter (clot catcher) by study personnel. The clot catcher is part of the single use canister for the Penumbra Aspiration Pump.

After the specimen is collected into the RNA collection tube, it is recommended to fill the RNA Clot collection tube with the fluid in the canister prior to sealing.

Prior to thrombectomy, and during flushing of the diagnostic or guide catheter, approximately 10 mL of extracranial blood should be collected from the diagnostic or guide catheter. It is recommended to allow approximately 10-15ml waste while backflushing prior to obtaining the 10ml sample. 5 mL will be placed into each of the EDT purple top collection tubes provided in the specimen collection kit.

5.5.4 Specimen for Minimally Invasive Surgery

Specimen will be collected during the procedure in the canister within the Penumbra Aspiration Pump by study personnel.

The specimen may be obtained in whole piece(s) or fragments after evacuation by the Artemis device. After the specimen is collected into the RNA collection tube it is recommended to fill the RNA Clot collection tube with the fluid in the canister prior to sealing.

Approximately 10 mL of blood should be collected from the arterial line and 5 mL will be placed into each of the EDT purple top collection tubes provided in the specimen collection kit The blood should be collected from the arterial line around the same time as the clot evacuation.

5.6 Discharge or 7 Days (whichever occurs first)

The following assessments will be performed per site routine practice at discharge or 7 Days post procedure:

- National Institutes of Health Stroke Scale (NIHSS)
- Modified Rankin Scale (mRS)
- Glasgow Coma Score (GCS) – (MIS only)
- SAE collection

5.7 90 Day Follow-Up ±14 Days (Optional phone visit)

The following assessment will be performed per site routine practice at this optional phone visit:

- Modified Rankin Scale (mRS)

Table 1: Schedule of Assessments

ACTIVITY	Baseline Evaluation	Procedure	Discharge or 7 Days[6]	90 Day Follow-Up[1] (±14 Days)
Informed Consent[2]	X			
Medical History	X			
Demographics	X			
Medication Review	X	X		
Clot Retrieval		X		
Blood Sample Collection		X		
Hematoma Volume[3]		X		
NIHSS	X		X	
mTICI[4]		X		
mRS	X[5]		X	X
GCS[3]	X		X	
Serious Adverse Events[7]		X	X	

[1] Optional Visit (conducted by phone)
[2] May be obtained up to 1 calendar day post-procedure and must be obtained prior to shipment of samples
[3] Minimally invasive surgery / hematoma evacuation patients only
[4] Ischemic stroke patients from digital subtracted angiography
[5] Historical (pre-stroke)
[6] Whichever occurs first
[7] The following SAEs will be collected through 7 days post procedure: Device related events, procedural related events, Symptomatic ICH (sICH) at 24 hours, symptomatic embolization of new territory (ENT) at end of procedure

6. Specimen Collection and Shipment

All specimen should be placed within the provided collection tube(s) then securely packaged in the specimen collection kit provided, and shipped to the Core Lab(s) as quickly as possible, but no later than 72 hours post procedure and after informed consent is obtained.

The study sponsor will provide specimen kits, comprised of the following components:

- Mini Sample collection tube(s) for the clot
- Sealable plastic biohazard bags
- Absorbent material
- Paraffin tape for mini sample collection tubes(s)
- Purple collection tubes for blood samples (x2)
- Return packaging for specimen shipment to the Core Lab(s)
- UN 3373, Biological substances, Category B label
- Instructions for specimen collection, packing and shipping the specimen kit

Prior to return shipment to the Core Lab(s), document the kit number for entry into the database. The samples should be packaged per the packaging and shipping instructions and must comply with the Department of Transportation (DOT), Hazardous Materials Regulations (HMR), 49 CFR Parts 171-180) and the International Airline Transport Associate (IATA) for shipping of Hazardous Materials.

Study personnel responsible for packaging and/or shipping must be trained in the packaging and shipping of Category B, biological substance, as described in the DOT/IATA division 6.2 and HMR 173.134(b)(10). Certification of training will be required for all study personnel delegated to perform specimen packaging and shipping as described in this protocol.

6.1 Specimen Destruction

Specimen collected as part of this registry may be destroyed for the following reasons:

- Requested by the sponsor according to the Core Lab Charter
- Requested in writing by a patient who has withdrawn their consent to participate
- Informed Consent not obtained as specified in section 5.4 Informed Consent

7. Investigator Responsibilities

7.1 Institutional Review Board Approval

Prior to enrolling patients into the study, the Investigator will ensure that proper Institutional Review Board (IRB) approval is obtained in accordance with applicable local state and federal laws and regulations. The IRB shall approve all study documents as appropriate, including but not limited to: the final protocol, amendments to the protocol, Instructions for Use (where applicable), and the informed consent.

The Investigator will report to the Sponsor or designee immediately if the approval to conduct the investigation is withdrawn by the IRB. The report will include a complete description of the reason(s) for which approval was withdrawn.

7.2 Informed Consent

The Investigator is responsible for ensuring that a signed and dated informed consent is obtained in accordance with Section 5.4 of this protocol and according to country and local requirements prior to conducting any study-related assessments and prior to enrollment of patients in the study.

7.3 Adherence to Protocol/Amendments and Applicable Law

The Investigator is responsible for overseeing, ensuring that the study is conducted, and completing the study according to this protocol and in accordance with the relevant aspects of I.S. EN ISO 14155:2020, Declaration of Helsinki, along with any conditions imposed by the reviewing Institutional Review Board (IRB), and all other applicable regulations. The Investigator shall approve and adhere to this protocol and any amendments that arise during the course of the study.

It is the Investigator’s responsibility to ensure that the staff assisting with the study have the appropriate qualifications, are fully instructed on the study procedures, and will respect study confidentiality.

7.4 Case Report Form Completion

The Investigator and study staff shall complete the case report forms (CRFs) associated with this study. Patient numbers shall be used to identify individual participants in this study. The CRFs should be a complete and accurate record of patient data collected during the study according to relevant aspects of I.S. EN ISO 14155, 21 CFR 11, Electronic Records; Electronic Signatures, and Good Clinical Practices (GCP) requirements. It is the Investigator's responsibility to ensure the quality of the data collected and recorded is appropriate and collected in accordance with GCP and all applicable regulations. Data entry will be performed by the study site(s). Investigators are responsible for completion and timely submission of data to Penumbra, Inc. Every reasonable effort should be made to complete data entry within 7 days of data collection.

7.5 Reporting

The Investigator will be responsible for reporting the following:

7.5.1 Adverse Events

Serious Adverse Events (AE) will be collected in this registry on patients treated with any of the RED reperfusion catheters from procedure through discharge. Serious device related events, serious procedure related adverse events, symptomatic ICH at 24 hours, device deficiencies leading to adverse events, and symptomatic embolization of new territory (ENT) and will be collected on the case report form through 7 days post procedure. No additional risk is associated with specimen collection.

7.5.2 Device Deficiencies

Any device deficiency that might lead to serious adverse events if appropriate action had not been taken, intervention had not occurred or circumstances had been less fortunate will be reported in EDC. All device deficiencies related to the identity, quality, durability, reliability, or performance of the devices used shall be documented and reported through the post market reporting process.

7.5.3 Protocol Deviation

Deviations defined in this protocol should be clearly documented, if identified during monitoring or through other means. For this study, deviations should be reported for the following categories:

- Inclusion/exclusion criteria deviation(s)
- Informed Consent deviation(s)

7.6 Records Retention

The Investigator shall maintain the records associated with this study for a period of at least two years after the date on which the investigation is completed. An eTMF will be used as the master repository for all site and Sponsor regulatory documents. These records include the following:

- Correspondence with the Sponsor or designee, and other Investigators
- Accountability records of receipt, use, and disposition of all study materials
- Patient source records, including but not limited to: Informed Consent Forms, copies of all completed CRFs, and supporting documents (laboratory reports and reports of diagnostic tests, medical records, etc.)
- All versions of study protocol
- Documentation of protocol deviations
- A copy of all approvals related to the clinical investigation
- The approved, blank, informed consent form

- All approval/acknowledgment letters from the IRB for all versions of the study protocol, ICF and other documents
- Clinical Trial Agreement(s)
- Signed and dated curriculum vitae for all study personnel
- Medical licenses for the Principal Investigator and all participating Sub-Investigators
- Financial disclosure for the Principal Investigator and all participating Sub-Investigators
- All required regulatory documents such as Delegation of Authority and training logs
- Signed Protocol Signature Page(s)

8. Sponsor Responsibilities

8.1 Training

The Sponsor is responsible for providing training on the protocol, Core Lab(s) procedures, and eCRF completion as applicable for all study staff per delegation of authority log.

8.2 Investigator List

The Sponsor shall keep a list of the names and addresses of the clinical Investigators for the study.

8.3 Data Monitoring

Penumbra is responsible for ensuring that the study is conducted according to the appropriate regulations.

- Guideline for Good Clinical Practice, (ICH E6)
- Clinical investigation of medical devices for human subjects – Good clinical practice (I.S. EN ISO 14155:2020)
- US Food and Drug Administration 21 CFR Title 21 Parts §11, §50, §54, §56, and §812

A Penumbra employee or designee will conduct the following site visits:

8.3.1 Site Qualification

Conducted to ensure the study site has the appropriate staff, facilities, and expertise to participate in the study. Site Qualification can be waived under certain circumstances.

8.3.2 Site Initiation

Conducted to train the investigational staff on CRF completion, Core Lab(s) processes, study requirements, and other relevant training.

8.3.3 Interim Monitoring

Conducted as needed to ensure the study site is operating in compliance with this protocol, continues to have the appropriate staff and facilities, and is correctly completing the CRFs.

The visits ensure that Investigators and their staff understand and accept their defined responsibilities, the Sponsor will maintain regular correspondence and perform periodic site visits during the course of the study to verify the continued acceptability of the facilities, compliance with the investigational plan, and maintenance of complete records. Clinical monitoring will include review of informed consent forms for enrolled patients. Informed consent, eCRFs and medical records for all enrolled patients will be made available to the Sponsor for review and collection.

8.3.4 Site Close Out

Conducted to ensure all study and regulatory-related activities have been completed prior to site closure.

8.4 Data Management

eCRFs will be used at all study sites. All study data will be entered into commercially available web-based electronic data capture system (EDC). Data entry will be performed by the study site personnel. Investigators are responsible for completion and timely submission of the data to the Sponsor. Every reasonable effort should be made to complete data entry within 7 days of data collection. This EDC system requires no on-site software installation or specific hardware to operate. Investigators, clinical coordinators, data managers, Core Lab personnel, and Penumbra clinical personnel access project information and study data centrally via a web browser.

Automated data quality checks will display warnings for invalid data. Additionally, manual review of data listings may be used to identify data discrepancies or inconsistencies. The study site may be queried for clarification concerning CRF discrepancies or inconsistencies identified. If CRF corrections are necessary, they will be made by the Investigator or an authorized member of the Investigator's staff that is delegated to the CRF/EDC. Questions or problems with submitted data will be addressed with the Principal Investigator via an electronic querying system, or through direct contact. The Investigator will review the CRFs for completeness and accuracy and provide his/her electronic signature and date to CRFs as evidence thereof. Any data items that have been changed will require reapplication of the electronic signature.

Study personnel will have an individual login and password to access the clinical study information based upon each individual's roles and responsibilities. The application provides hierarchical user permission data entry, viewing, and reporting options.

All data entry and data update information, including the date and person performing the action, will be available via the audit trail, which is part of the EDC system.

All eCRFs and data files will be secured to ensure confidentiality. Investigators are required to maintain source documents required by the protocol, including laboratory results, reports, supporting medical records, and Informed Consent Forms. The source documents will be used during the regular monitoring visits to verify information entered on the CRFs.

9. Ethical Requirements

9.1 Declaration of Helsinki

The study will be performed in accordance with the applicable aspects I.S. EN ISO 14155, recommendations guiding physicians in biomedical research involving human subjects adopted by the 18th World Medical Assembly, Helsinki, Finland (1964 and later revisions), ICH, and US FDA GCP guidelines.

It is the responsibility of the Investigator to obtain approval of the study protocol from the Institutional Review Board (IRB) and to keep the IRB informed of any amendments to the protocol and informed consent forms. All correspondence with the IRB should be filed by the Investigator and copies sent to the Sponsor or its designee.

9.2 Informed Consent

The Investigator is responsible for ensuring that a completed informed consent is obtained in accordance with Section 5.4 of this protocol, as delegated by the site-specific Delegation of Authority, and according to country and local requirements.

9.3 Patient Data Protection

Each patient will be assigned a unique patient identification number at the time of enrollment. This patient identification number will be retained throughout the study. Study sites will keep a log that notes the patient's name and corresponding patient identification number. All case report forms (CRFs) will be tracked, evaluated, and stored using only the patient ID number. No personal identifying information will be included on the case report forms.

The informed consent form will notify patients that study monitors, auditors, and representatives of government agencies and ethics committees will have access to

personal identifying information to ensure that data reported on the CRFs corresponds to the person who signed the consent form and the information contained in the source documentation.

10. Statistical Procedures

10.1 General Statistical Considerations

All statistical analyses will be descriptive in nature. Descriptive statistics will include the number of observations, mean, median, standard deviation, inter-quartile range, minimum and maximum for continuous variables and counts and percentages for discrete variables. All confidence intervals presented will be two-sided. Analyses will be conducted using SAS (SAS Institute, Cary, NC). The specific details of the planned analyses are described completely in the statistical analysis plan.

10.2 Sample Size Estimation

Due to the descriptive nature of this registry, no formal sample size calculations were performed. Approximately up to 400 patients will be enrolled in the registry.

10.3 Control of Systematic Error and Bias

The registry will be conducted under a common protocol for each study site with the intention of pooling the data for analysis. Every effort will be made to promote consistency in study execution at each study site.

10.4 Missing Data and Imputation Methods

Every effort is to be made to keep all missing data to a minimum. Despite the clinical site's best efforts, some missing data may be inevitable mainly due to lost-to-follow-up. (LTFU)

10.5 Definition of Populations

10.5.1 Enrolled

Patients will be considered enrolled upon signed informed consent and specimen collection.

10.5.2 Completed

All patients who were enrolled and completed the registry follow-up or were known to have died prior to the follow-up timepoint.

10.5.3 Early Termination

Patients who were enrolled but did not complete follow-up and were not known to have died.

10.6 Definition of Analysis Population

All enrolled patients will be analyzed under the intent-to-treat (ITT) principle.

10.7 Interim Analysis

No interim analyses are planned for the purpose of stopping the study early. Interim analysis may be performed for publication of registry results. No adjustments will be made to the confidence bounds for the final analysis.

10.8 Statistical Analysis

Clot characteristics, such as clot location, clot organization, composition, source of thrombus, and clot dimensions will be summarized using descriptive statistics.

Baseline data including, but not limited to demographics, clinical, procedural, and imaging characteristics from ischemic stroke patients and patients eligible for minimally invasive hematoma evacuation will be summarized using descriptive statistics.

Sub-group analyses will be performed to examine patient outcomes based on initial presentations and radiologic characteristics. Exploratory multivariate analyses will be conducted.

Results collected at multiple visits will be summarized at each visit for which they are collected as described Schedule of Assessments Table. Summaries for all measures will include all observed data for each visit.

10.9 Pooling Across Centers

Analyses will be present using data pooled across centers. Descriptive statistics for key variables will be presented to assess any potential site effects.

10.10 Final Report

A final report will be completed, even if the study is prematurely terminated. At the conclusion of the registry, a multi-center abstract reporting the results will be prepared and may be presented at a major meeting(s). A multi-center publication may also be prepared for publication in a reputable scientific journal. The publication of results from any single center experience within the registry is not allowed until the aggregate study results have been published, unless there is written consent from the Sponsor.

11. Study Committees: Core Lab(s)

11.1 Central Core and Pathophysiology Lab

Up to three independent laboratories will follow the Study Core Lab Charter to analyze collected samples via histology, proteomics, and RNA sequencing. The Core Lab(s) will also be responsible for appropriate sample storage and destruction.

12. Study Administration

12.1 Clinical Trial Termination/Withdrawal

Patients may be terminated or withdrawn from the study for the following reasons:

- Voluntary withdrawal of consent— meaning that a patient voluntarily chooses not to participate further in the study. All data collected up to the withdrawal of consent will be maintained in the study database. Withdrawn patients will not have any additional follow-up and will not be replaced.
- Patients may also be withdrawn at the Investigator's discretion if within their best interest. A patient's participation in the clinical study will be terminated if the Investigator believes that this is in the patient's best medical interest or if the patient no longer complies with the clinical study requirements.
- Upon withdrawal of consent, all specimens collected prior to receipt of notice of withdrawal will not be destroyed and will remain part of this registry and stored for future research by Sponsor unless written notification from the patient is received requesting that all samples are to be destroyed, as specified in Section 6.1 Specimen Destruction.

The sponsor may temporarily suspend or prematurely terminate the study at any time for the following reasons:

- Suspicion of risk to patients
- If no positive IRB decision is obtained or if the judgement of the IRB is revoked
- If the applicable regulatory body has made an irrevocable objection
- If it transpires that continuation of study cannot serve any scientific purpose, and this is confirmed by the IRB
- Business reasons

The Sponsor will document reasons for study suspension or premature termination and notify the PIs. The Sponsor will ensure that the IRB and regulatory authorities are notified in a timely manner.

The Sponsor will continue to provide resources to fulfil the obligations from the study protocol and existing agreements for following up the patients enrolled in the study.

The Principal Investigators will promptly inform the enrolled patients at his/her site, if appropriate.

If the Sponsor temporarily suspends the study and wishes to resume it, the Sponsor will inform the PIs, IRB, and (if appropriate) regulatory authorities. The Sponsor

will provide a rationale for resuming the study. IRB must provide written approval before the study is resumed.

12.2 Protocol Adherence and Amendments

Prior to beginning the study, the Principal Investigator must sign the Protocol Signature Page documenting his/her agreement to conduct the study in accordance with this protocol. Deviations outlined in section 6.5.1 must be documented and reported to Penumbra as soon as possible, and to the IRB per local guidelines and government regulations.

12.3 Trial Registration

The study will be registered in a publicly accessible trial database (e.g., clinical trials.gov) prior to study initiation.

13. Publication of Information

All information and data generated in association with this study will be held in strict confidence and remain the sole property of the Sponsor. The Investigator agrees to use this information for the sole purpose of completing this study and for no other purpose without written consent from the Sponsor.

The results of this study may be offered for publication. The Investigators and the Sponsor shall collaborate in the writing of the study to ensure accuracy. All information not previously published concerning the research, including patent applications, manufacturing processes, basic scientific data, etc., is considered confidential and should remain the sole property of Penumbra. The Investigator agrees to use this information only in connection with this study and will not use it for other purposes without written permission from the Sponsor.

14. Contact Information

The address of Penumbra Incorporated is:

Penumbra, Inc.
One Penumbra Place
Alameda, CA 94502
Tel. (510) 748-3200
Fax (510) 814-8305

Key contacts for the Study include:

[redacted] [redacted] [redacted]

15. References

1. Johnson, W., Onuma, O., Owolabi, M., and Sachdev, S. (2016). Stroke: A global response is needed. *Bull World Health Organ*. 94(9): p. 634-634A.
2. Benjamin, E.J., Blaha, M.J., Chiuve, S.E., Cushman, M., Das, S.R., Deo, R., . . . Stroke Statistics, S. (2017). Heart disease and stroke statistics-2017 update: A report from the american heart association. *Circulation*. 135(10): p. e146-e603.
3. Powers, W.J., Rabinstein, A.A., Ackerson, T., Adeoye, O.M., Bambakidis, N.C., Becker, K., . . . American Heart Association Stroke, C. (2018). 2018 guidelines for the early management of patients with acute ischemic stroke: A guideline for healthcare professionals from the american heart association/american stroke association. *Stroke*. 49(3): p. e46-e110.
4. Turk, A.S., 3rd, Siddiqui, A., Fifi, J.T., De Leacy, R.A., Fiorella, D.J., Gu, E., . . . Mocco, J. (2019). Aspiration thrombectomy versus stent retriever thrombectomy as first-line approach for large vessel occlusion (compass): A multicentre, randomised, open label, blinded outcome, non-inferiority trial. *Lancet*. 393(10175): p. 998-1008.
5. Hemphill, J.C., 3rd, Bonovich, D.C., Besmertis, L., Manley, G.T., and Johnston, S.C. (2001). The ich score: A simple, reliable grading scale for intracerebral hemorrhage. *Stroke*. 32(4): p. 891-7.
6. Goyal, N., Tsivgoulis, G., Malhotra, K., Katsanos, A.H., Pandhi, A., Alsherbini, K.A., . . . Arthur, A.S. (2019). Minimally invasive endoscopic hematoma evacuation vs best medical management for spontaneous basal-ganglia intracerebral hemorrhage. *J Neurointerv Surg*.
7. Hanley, D.F., Lane, K., McBee, N., Ziai, W., Tuhrim, S., Lees, K.R., . . . Investigators, C.I. (2017). Thrombolytic removal of intraventricular haemorrhage in treatment of severe stroke: Results of the randomised, multicentre, multiregion, placebo-controlled clear iii trial. *Lancet*. 389(10069): p. 603-611.
8. Hanley, D.F., Thompson, R.E., Rosenblum, M., Yenokyan, G., Lane, K., McBee, N., . . . Investigators, M.I. (2019). Efficacy and safety of minimally invasive surgery with thrombolysis in intracerebral haemorrhage evacuation (mistie iii): A randomised, controlled, open-label, blinded endpoint phase 3 trial. *Lancet*. 393(10175): p. 1021-1032.
9. Spiotta, A.M., Fiorella, D., Vargas, J., Khalessi, A., Hoit, D., Arthur, A., . . . Turner, R.D. (2015). Initial multicenter technical experience with the apollo device for minimally invasive intracerebral hematoma evacuation. *Neurosurgery*. 11 Suppl 2: p. 243-51; discussion 251.
10. Feigin, V.L., Krishnamurthi, R.V., Parmar, P., Norrving, B., Mensah, G.A., Bennett, D.A., . . . Group, G.B.D.S.P.E. (2015). Update on the global burden of ischemic and hemorrhagic stroke in 1990-2013: The gbd 2013 study. *Neuroepidemiology*. 45(3): p. 161-76.
11. Alawieh, A., Vargas, J., Fargen, K.M., Langley, E.F., Starke, R.M., De Leacy, R., . . . Spiotta, A.M. (2019). Impact of procedure time on outcomes of thrombectomy for stroke. *J Am Coll Cardiol*. 73(8): p. 879-890.

12. De Meyer, S.F., Andersson, T., Baxter, B., Bendszus, M., Brouwer, P., Brinjikji, W., . . . Clot Summit, G. (2017). Analyses of thrombi in acute ischemic stroke: A consensus statement on current knowledge and future directions. *Int J Stroke*. 12(6): p. 606-614.
13. Brouwer, P.A., Brinjikji, W., and De Meyer, S.F. (2018). Clot pathophysiology: Why is it clinically important? *Neuroimaging Clin N Am*. 28(4): p. 611-623.
14. Czaplicki, C., Albadawi, H., Partovi, S., Gandhi, R.T., Quencer, K., Deipolyi, A.R., and Oklu, R. (2017). Can thrombus age guide thrombolytic therapy? *Cardiovasc Diagn Ther*. 7(Suppl 3): p. S186-S196.
15. Sporns, P.B., Hanning, U., Schwindt, W., Velasco, A., Buerke, B., Cnyrim, C., . . . Niederstadt, T. (2017). Ischemic stroke: Histological thrombus composition and pre-interventional ct attenuation are associated with intervention time and rate of secondary embolism. *Cerebrovasc Dis*. 44(5-6): p. 344-350.
16. Kim, S.K., Yoon, W., Kim, T.S., Kim, H.S., Heo, T.W., and Park, M.S. (2015). Histologic analysis of retrieved clots in acute ischemic stroke: Correlation with stroke etiology and gradient-echo mri. *AJNR Am J Neuroradiol*. 36(9): p. 1756-62.
17. Marder, V.J., Chute, D.J., Starkman, S., Abolian, A.M., Kidwell, C., Liebeskind, D., . . . Saver, J.L. (2006). Analysis of thrombi retrieved from cerebral arteries of patients with acute ischemic stroke. *Stroke*. 37(8): p. 2086-93.
18. Boeckh-Behrens, T., Schubert, M., Forschler, A., Prothmann, S., Kreiser, K., Zimmer, C., . . . Poppert, H. (2016). The impact of histological clot composition in embolic stroke. *Clin Neuroradiol*. 26(2): p. 189-97.
19. Mehta, B.P. and Nogueira, R.G. (2012). Should clot composition affect choice of endovascular therapy? *Neurology*. 79(13 Suppl 1): p. S63-7.
20. Sumaya, W., Wallentin, L., James, S.K., Siegbahn, A., Gabrysch, K., Bertilsson, M., . . . Storey, R.F. (2018). Fibrin clot properties independently predict adverse clinical outcome following acute coronary syndrome: A plato substudy. *Eur Heart J*. 39(13): p. 1078-1085.
21. Hashimoto, T., Hayakawa, M., Funatsu, N., Yamagami, H., Satow, T., Takahashi, J.C., . . . Toyoda, K. (2016). Histopathologic analysis of retrieved thrombi associated with successful reperfusion after acute stroke thrombectomy. *Stroke*. 47(12): p. 3035-3037.
22. Maekawa, K., Shibata, M., Nakajima, H., Mizutani, A., Kitano, Y., Seguchi, M., . . . Miya, F. (2018). Erythrocyte-rich thrombus is associated with reduced number of maneuvers and procedure time in patients with acute ischemic stroke undergoing mechanical thrombectomy. *Cerebrovasc Dis Extra*. 8(1): p. 39-49.
23. Shin, J.W., Jeong, H.S., Kwon, H.J., Song, K.S., and Kim, J. (2018). High red blood cell composition in clots is associated with successful recanalization during intra-arterial thrombectomy. *PLoS One*. 13(5): p. e0197492.
24. Drabik, L., Wolkow, P., and Undas, A. (2017). Fibrin clot permeability as a predictor of stroke and bleeding in anticoagulated patients with atrial fibrillation. *Stroke*. 48(10): p. 2716-2722.
25. Maatman, B.T., Schmeisser, G., and Kreutz, R.P. (2018). Fibrin clot strength in patients with diabetes mellitus measured by thrombelastography. *J Diabetes Res*. 2018: p. 4543065.
26. Undas, A. and Zabczyk, M. (2018). Antithrombotic medications and their impact on fibrin clot structure and function. *J Physiol Pharmacol*. 69(4).

27. Banks, J.L. and Marotta, C.A. (2007). Outcomes validity and reliability of the modified rankin scale: Implications for stroke clinical trials: A literature review and synthesis. *Stroke*. 38(3): p. 1091-6.
28. Moore, L., Lavoie, A., Camden, S., Le Sage, N., Sampalis, J.S., Bergeron, E., and Abdous, B. (2006). Statistical validation of the glasgow coma score. *J Trauma*. 60(6): p. 1238-43; discussion 1243-4.
29. Meyer, B.C., Hemmen, T.M., Jackson, C.M., and Lyden, P.D. (2002). Modified national institutes of health stroke scale for use in stroke clinical trials: Prospective reliability and validity. *Stroke*. 33(5): p. 1261-6.

16. Appendix

16.1 Modified Rankin Scale [27]

0	No symptoms
1	No significant disability, despite symptoms; able to perform all usual duties and activities
2	Slight disability; unable to perform all previous activities but able to look after own affairs without assistance
3	Moderate disability; requires some help, but able to walk without assistance
4	Moderately severe disability; unable to walk without assistance and unable to attend to own bodily needs without assistance
5	Severe disability; bedridden, incontinent, and requires constant nursing care and attention
6	Death

16.2 Glasgow Coma Scale [28]

Glasgow Coma Score		
Eye Opening (E)	Verbal Response (V)	Motor Response (M)
4= Open before stimulus (Rating=Spontaneous) 3= After spoken or shouted request (Rating=To sound) 2= After fingertip stimulus (Rating=To pressure) 1= No opening at any time, no interfering factor (Rating=None)	5= Correctly gives name, place and date (Rating=Orientated) 4= Not oriented but communicates coherently (Rating=Confused) 3= Intelligible single words (Rating=Words) 2= Only moans/groan (Rating=Sounds) 1= No audible response, no interfering factor (Rating=None)	6= Obey 2-part request (Rating=Obeys commands) 5= Brings hand above clavicle to stimulus on head/neck (Rating=Localizing) 4= Bends arm at elbow rapidly but features not predominantly abnormal (Rating=Normal flexion) 3= Bends arm at elbow, features clearly predominantly abnormal (Rating=Abnormal flexion) 2= Extends arm at elbow (Rating=Extension) 1= No movement in arms/legs, no interfering factor (Rating=None)
		Total= E+V+M

16.3 National Institute of Health Stroke Scale [29]

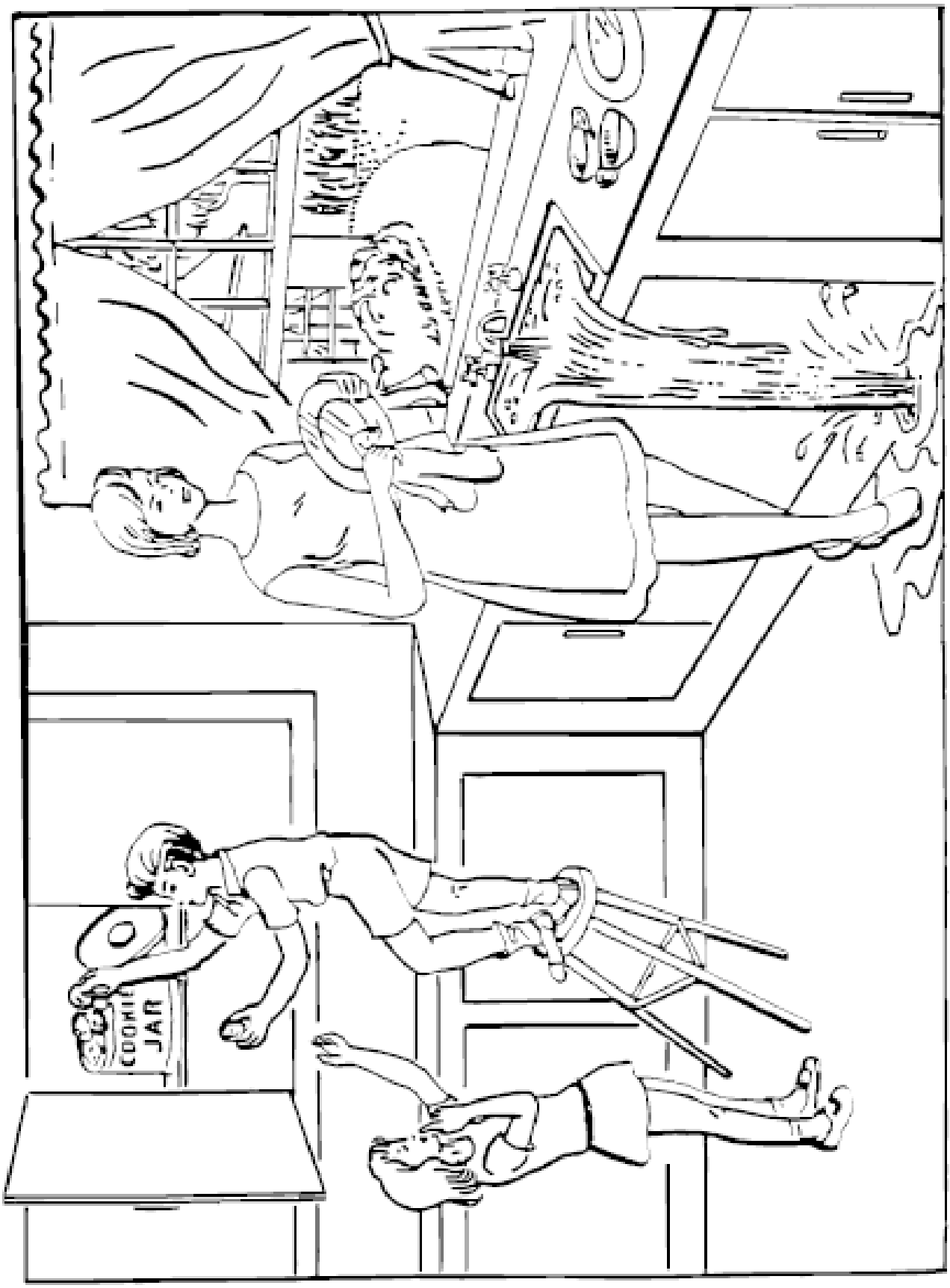
NIH STROKE SCALE		
Instructions	Scale Definition	Score
1a. Level of Consciousness: The investigator must choose a response if a full evaluation is prevented by such obstacles as an endotracheal tube, language barrier, orotracheal trauma/bandages. A 3 is scored only if the patient makes no movement (other than reflexive posturing) in response to noxious stimulation.	0 = Alert; keenly responsive. 1 = Not alert; but arousable by minor stimulation to obey, answer, or respond. 2 = Not alert; requires repeated stimulation to attend, or is obtunded and requires strong or painful stimulation to make movements (not stereotyped). 3 = Responds only with reflex motor or autonomic effects or totally unresponsive, flaccid, and areflexic.	____
1b. LOC Questions: The patient is asked the month and his/her age. The answer must be correct - there is no partial credit for being close. Aphasic and stuporous patients who do not comprehend the questions will score 2. Patients unable to speak because of endotracheal intubation, orotracheal trauma, severe dysarthria from any cause, language barrier, or any other problem not secondary to aphasia are given a 1. It is important that only the initial answer be graded and that the examiner not "help" the patient with verbal or non-verbal cues.	0 = Answers both questions correctly. 1 = Answers one question correctly. 2 = Answers neither question correctly.	____
1c. LOC Commands: The patient is asked to open and close the eyes and then to grip and release the non-paretic hand. Substitute another one step command if the hands cannot be used. Credit is given if an unequivocal attempt is made but not completed due to weakness. If the patient does not respond to command, the task should be demonstrated to him or her (pantomime), and the result scored (i.e., follows none, one or two commands). Patients with trauma, amputation, or other physical impediments should be given suitable one-step commands. Only the first attempt is scored.	0 = Performs both tasks correctly. 1 = Performs one task correctly. 2 = Performs neither task correctly.	____

NIH STROKE SCALE		
2. Best Gaze: Only horizontal eye movements will be tested. Voluntary or reflexive (oculocephalic) eye movements will be scored, but caloric testing is not done. If the patient has a conjugate deviation of the eyes that can be overcome by voluntary or reflexive activity, the score will be 1. If a patient has an isolated peripheral nerve paresis (CN III, IV or VI), score a 1. Gaze is testable in all aphasic patients. Patients with ocular trauma, bandages, pre-existing blindness, or other disorder of visual acuity or fields should be tested with reflexive movements, and a choice made by the investigator. Establishing eye contact and then moving about the patient from side to side will occasionally clarify the presence of a partial gaze palsy.	0 = Normal. 1 = Partial gaze palsy; gaze is abnormal in one or both eyes, but forced deviation or total gaze paresis is not present. 2 = Forced deviation, or total gaze paresis not overcome by the oculocephalic maneuver.	____
3. Visual: Visual fields (upper and lower quadrants) are tested by confrontation, using finger counting or visual threat, as appropriate. Patients may be encouraged, but if they look at the side of the moving fingers appropriately, this can be scored as normal. If there is unilateral blindness or enucleation, visual fields in the remaining eye are scored. Score 1 only if a clear-cut asymmetry, including quadrantanopia, is found. If patient is blind from any cause, score 3. Double simultaneous stimulation is performed at this point. If there is extinction, patient receives a 1, and the results are used to respond to item 11.	0 = No visual loss. 1 = Partial hemianopia. 2 = Complete hemianopia. 3 = Bilateral hemianopia (blind including cortical blindness).	____
4. Facial Palsy: Ask – or use pantomime to encourage – the patient to show teeth or raise eyebrows and close eyes. Score symmetry of grimace in response to noxious stimuli in the poorly responsive or non-comprehending patient. If facial trauma/bandages, orotracheal tube, tape or other physical barriers obscure the face, these should be removed to the extent possible.	0 = Normal symmetrical movement. 1 = Minor paralysis (flattened nasolabial fold, asymmetry on smiling). 2 = Partial paralysis (total or near-total paralysis of lower face). 3 = Complete paralysis of one or both sides (absence of facial movement in the upper and lower face).	____

NIH STROKE SCALE		
5. Motor Arm: The limb is placed in the appropriate position: extend the arms (palms down) 90 degrees (if sitting) or 45 degrees (if supine). Drift is scored if the arm falls before 10 seconds. The aphasic patient is encouraged using urgency in the voice and pantomime, but not noxious stimulation. Each limb is tested in turn, beginning with the non-paretic arm. Only in the case of amputation or joint fusion at the shoulder, the examiner should record the score as untestable (UN), and clearly write the explanation for this choice.	0 = No drift; limb holds 90 (or 45) degrees for full 10 seconds. 1 = Drift; Limb holds 90 (or 45) degrees, but drifts down before full 10 seconds: does not hit bed or other support. 2 = Some effort against gravity; limb cannot get to or maintain (if cued) 90 (or 45) degrees, drifts down to bed, but has some effort against gravity. 3 = No effort against gravity; limb falls. 4 = No movement. UN = Amputation or joint fusion, explain:	
	5a. Left Arm 5b. Right Arm	____
6. Motor Leg: The limb is placed in the appropriate position: hold the leg at 30 degrees (always tested supine). Drift is scored if the leg falls before 5 seconds. The aphasic patient is encouraged using urgency in the voice and pantomime, but not noxious stimulation. Each limb is tested in turn, beginning with the non-paretic leg. Only in the case of amputation or joint fusion at the hip, the examiner should record the score as untestable (UN), and clearly write the explanation for this choice.	0 = No drift; leg holds 30 degrees position for full 5 seconds. 1 = Drift; leg falls by the end of the 5 second period but does not hit bed. 2 = Some effort against gravity; leg falls to bed by 5 seconds, but has some effort against gravity. 3 = No effort against gravity; leg falls to bed immediately. 4 = No movement. UN = Amputation or joint fusion, explain: _____ 6a. Left Leg 6b. Right Leg	____
7. Limb Ataxia: This item is aimed at finding evidence of a unilateral cerebellar lesion. Test with eyes open. In case of visual defect, ensure testing is done in intact visual field. The finger-nose-finger and heel-shin tests are performed on both sides, and ataxia is scored only if present out of proportion to weakness. Ataxia is absent in the patient who cannot understand or is paralyzed. Only in the case of amputation or joint fusion, the examiner should record the score as untestable (UN), and clearly write the	0 = Absent. 1 = Present in one limb. 2 = Present in two limbs. UN = Amputation or joint fusion, explain: _____	____

NIH STROKE SCALE		
explanation for this choice. In case of blindness, test by having the patient touch nose from extended arm position.		
8. Sensory: Sensation or grimace to pinprick when tested, or withdrawal from noxious stimulus in the obtunded or aphasic patient. Only sensory loss attributed to stroke is scored as abnormal and the examiner should test as many body areas (arms [not hands], legs, trunk, face) as needed to accurately check for hemisensory loss. A score of 2, "severe or total sensory loss," should only be given when a severe or total loss of sensation can be clearly demonstrated. Stuporous and aphasic patients will, therefore, probably score 1 or 0. The patient with brainstem stroke who has bilateral loss of sensation is scored 2. If the patient does not respond and is quadriplegic, score 2. Patients in a coma (item 1a=3) are automatically given a 2 on this item.	0 = Normal; no sensory loss. 1 = Mild to moderate sensory loss; patient feels pinprick is less sharp or is dull on the affected side; or there is a loss of superficial pain with pinprick, but patient is aware of being touched. 2 = Severe to total sensory loss; patient is not aware of being touched in the face, arm, and leg.	____
9. Best Language: A great deal of information about comprehension will be obtained during the preceding sections of the examination. For this scale item, the patient is asked to describe what is happening in the attached picture, to name the items on the attached naming sheet and to read from the attached list of sentences. Comprehension is judged from responses here, as well as to all of the commands in the preceding general neurological exam. If visual loss interferes with the tests, ask the patient to identify objects placed in the hand, repeat, and produce speech. The intubated patient should be asked to write. The patient in a coma (item 1a=3) will automatically score 3 on this item. The examiner must choose a score for the patient with stupor or limited cooperation, but a score of 3 should be used only if the patient is mute and follows no one-step commands.	0 = No aphasia; normal. 1 = Mild-to-moderate aphasia; some obvious loss of fluency or facility of comprehension, without significant limitation on ideas expressed or form of expression. Reduction of speech and/or comprehension, however, makes conversation about provided materials difficult or impossible. For example, in conversation about provided materials, examiner can identify picture or naming card content from patient's response. 2 = Severe aphasia; all communication is through fragmentary expression; great need for inference, questioning, and guessing by the listener. Range of information that can be exchanged is limited; listener carries burden of communication. Examiner cannot	____

NIH STROKE SCALE		
	identify materials provided from patient response. 3 = Mute, global aphasia; no usable speech or auditory comprehension.	
10. Dysarthria: If patient is thought to be normal, an adequate sample of speech must be obtained by asking patient to read or repeat words from the attached list. If the patient has severe aphasia, the clarity of articulation of spontaneous speech can be rated. Only if the patient is intubated or has other physical barriers to producing speech, the examiner should record the score as untestable (UN), and clearly write an explanation for this choice. Do not tell the patient why he or she is being tested.	0 = Normal. 1 = Mild to moderate dysarthria; patient slurs at least some words and, at worst, can be understood with some difficulty. 2 = Severe; patient's speech is so slurred as to be unintelligible in the absence of or out of proportion to any dysphasia, or is mute/anarthric. UN = Intubated or other physical barrier, explain:	____
11. Extinction and Inattention (formerly Neglect): Sufficient information to identify neglect may be obtained during the prior testing. If the patient has a severe visual loss preventing visual double simultaneous stimulation, and the cutaneous stimuli are normal, the score is normal. If the patient has aphasia but does appear to attend to both sides, the score is normal. The presence of visual spatial neglect or anosagnosia may also be taken as evidence of abnormality. Since the abnormality is scored only if present, the item is never untestable.	0 = No abnormality. 1 = Visual, tactile, auditory, spatial, or personal inattention or extinction to bilateral simultaneous stimulation in one of the sensory modalities. 2 = Profound hemi-inattention or extinction to more than one modality; does not recognize own hand or orients to only one side of space.	____


COOKIE
JAR

You know how.

Down to earth.

I got home from work.

Near the table in the dining room.

They heard him speak on the radio last night.

MAMA

TIP – TOP

FIFTY – FIFTY

THANKS

HUCKLEBERRY

BASEBALL PLAYER

16.4 Abbreviations

AE	Adverse Event
AIS	Acute Ischemic Stroke
CFR	Code of Federal Regulations
CRF	Case Report Form
DNA	Deoxyribonucleic Acid
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture
EN	European Norm
ENT	Embolization to Uninvolved or New Territories
FDA	Food and Drug Administration
FU	Follow-Up
GCP	Good Clinical Practices
GCS	Glasgow Coma Score
I/E	Inclusion/Exclusion
IA	Intra-Arterial
ICF	Informed Consent Form
IFU	Instructions For Use
IRB	Institutional Review Board
I.S.	Irish Standard
ISO	International Organization for Standardization
ITT	Intent To Treat
IV	Intravenous
LAR	Legally Authorized Representative
LTFU	Lost to Follow-Up
MIS	Minimally Invasive Surgery
mRS	Modified Rankin Scale
MT	Mechanical Thrombectomy
mTICI	Modified Treatment in Cerebral Ischemia
NIHSS	National Institute of Health Stroke Scale
PP	Per Protocol
RNA	Ribonucleic Acid
SAE	Serious Adverse Event
sICH	Symptomatic Intracerebral Hemorrhage; defined as 24 hour CT evidence of an ECASS defined ICH and a 4-point or more worsening of the NIHSS score
SNPs	Single Nucleotide Polymorphism
tPA	Tissue Plasminogen Activator
US	United States

16.5 Definitions

16.5.3 Adverse Event (AE): any untoward medical occurrence, unintended disease or injury, or untoward clinical signs (including abnormal laboratory findings) in participants, users or other persons, whether or not related to the study device and whether anticipated or unanticipated.

- **Serious Adverse Event (SAE)** An adverse event is serious if the event led to the following:
    - Death
    - Serious deterioration in the health of the participant that resulted in any of the following:
        - Life-threatening illness or injury
        - Permanent impairment of a body structure or a body function
        - Hospitalization or prolongation of patient hospitalization
        - Medical or surgical intervention to prevent life-threatening illness or injury or permanent impairment to a body structure or a body function
    - Fetal distress, fetal death or a congenital physical or mental impairment or birth defect
- **Unanticipated Adverse Device Event (UADE):** An unanticipated adverse device effect is any serious adverse effect on health or safety or any life-threatening problem or death caused by, or associated with, a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the protocol, IFU, or risk analysis. Unanticipated adverse device effect also includes any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of participants.
- **Relationship to the Device**
    - **Definite:** The temporal sequence is relevant and the event abates upon study device application completion/removal, or reappearance of the event on repeat study device application.
    - **Probable:** The temporal sequence is relevant or the AE abates upon study device application completion/removal or the AE cannot be reasonably explained by the participant's condition or comorbidities. The AE is related or most likely associated with the study device.
    - **Possible:** The temporal sequence between the study device and the AE is such that the relationship is not unlikely or there is no contradicting evidence that can reasonably explain the participant's condition. There is a possibility of a relationship between the AE and the study device.
    - **Unrelated:** The AE is not associated with the device. There is no relation between the AE and the study device.

Similar grading will be used for assessing the relationship to index procedure.

INSIGHT: Investigation of Clot in Ischemic Stroke and Hematoma Evacuation

STATISTICAL ANALYSIS PLAN

CLP-15131

Version 3.0

Sponsor

Penumbra, Inc.

One Penumbra Place

Alameda, CA 94502

USA

Contents

1. Overview

This is a prospective, multicenter, registry for the collection and analysis of specimen collected from ischemic stroke thrombectomy procedures and minimally invasive interventions for intracranial hematoma evacuation utilizing the Penumbra Aspiration Pump. The aim of the study is to collect and analyze specimen via histology, RNA sequencing, Single Nucleotide Polymorphisms (SNPs), and proteomic analysis. This will then be correlated to comprehensive data relating to patient's clinical presentation, hospital course, and length of hospital stay. Patients eligible for thrombectomy or minimally invasive surgery, who meet the inclusion and none of the exclusion criteria will be considered for enrollment. Treatment will be per site routine practice, using FDA cleared devices. Patients will be considered enrolled upon signed informed consent and specimen collection. Up to 400 patients from up to 30 sites in the United States will be enrolled into this registry. Each site will be limited to a maximum enrollment of 60 subjects (approximately 15% of the total enrolled). It is anticipated that enrollment will take approximately 2 years. Each patient will be in the registry for up to 90 days from enrollment to last follow-up. Data for each subject will be collected at the time of enrollment, discharge or 7days (whichever comes first) and 90 Days (optional) follow-up.

2. Study objectives

This study will examine whether clot composition, genetic, and proteomic variables can provide insight into thrombus etiology and/or have impact on clinical outcomes in patients with outwardly similar initial presentations.

2.1 Primary Objectives

The aim of the study is to collect and analyze specimen from, ischemic stroke patients undergoing thrombectomy procedures, and from patients undergoing minimally invasive surgery for intracranial hematoma evacuation. Clot and blood specimen will be analyzed via histology, RNA

sequencing, Single Nucleotide Polymorphisms (SNPs), and proteomic analysis.

2.2 Secondary Objectives

To prospectively collect and correlate data relating patient's clinical presentation, hospital course, and length of hospital stay to specimen histology, RNA sequencing, SNPs, and proteomic analysis.

3. Sample size

Due to the descriptive nature of this registry with no formal statistical hypothesis test, sample size calculations were not performed. Up to 400 subjects will be enrolled in the registry.

4. Interim analysis

No interim analyses are planned for the purpose of stopping the study early. Interim analysis may be performed for publication of registry results. No adjustments will be made to the confidence bounds for the final analysis.

5. General Statistical methods

No formal statistical testing is planned for this study. All statistical analyses will be descriptive in nature. Descriptive statistics will include the number of observations, mean, median, standard deviation, inter-quartile range, minimum and maximum for continuous variables and counts and percentages for discrete variables. All confidence intervals presented will be two-sided. Analyses will be conducted using SAS (SAS Institute, Cary, NC).

6. Patient Disposition

6.1 Enrolled

Patients will be considered enrolled upon signed informed consent and specimen collection.

6.2 Completed

All patients who were enrolled and completed either the Discharge/7 Day or 90 Day Follow-Up visit or were known to have died prior to 90 days are considered completed.

6.3 Early Termination

Subjects who were enrolled but did not complete any follow-up visits and were not known to have died.

7. Definition of Analysis Population

7.1 Intent to treat

All enrolled subjects will be analyzed under the intent-to-treat (ITT) principle.

8. Statistical Analysis

This plan is limited to the analysis of clinical data and specimen histology. The number of patients enrolled, patients completing the study, patients not completing the study, and patients completing the optional 90 Day Follow-Up visit will be summarized. Clot characteristics, such as clot location, clot organization, composition, clot etiology, clot dimensions, and specimen histology, will be summarized using descriptive statistics[1]. Baseline data including, but not limited to demographics, clinical, procedural, and imaging characteristics from ischemic stroke patients and patients eligible for minimally invasive hematoma evacuation will be summarized using descriptive statistics. Exploratory multivariate analyses will be conducted. Results collected at multiple

visits will be summarized at each visit for which they are collected as described in Schedule of Assessments Table. Summaries for all measures will include all observed data for each visit.

9. Analysis of adverse events

Serious adverse events will be collected in this registry on patients treated with any of the RED reperfusion catheters from procedure through discharge or 7 days post-procedure. Serious device related adverse events, serious procedure related adverse events will be summarized by showing the number and percent of subjects which report the event.

Other safety events collected on the case report form through 7 days post procedure will include symptomatic ICH at 24 hours, and symptomatic embolization of uninvolved or new territory (ENT)

All information pertaining to adverse events noted during the study will be listed by subject, detailing category, date of onset, date of resolution, relation to device or procedure and seriousness. The onset of serious adverse events will be shown relative (in number of days) to the day of procedure.

10. Subgroup analysis

To evaluate the impact of baseline conditions on procedural and clinical outcomes as well as clot characteristics, subgroup analyses will be performed for procedural outcomes of mTICI score, clinical outcomes of mortality at 90 days, NIHSS score at discharge and the proportion of patients that achieve mRS of 0 to 2 at 90 days (if available) and clot characteristics of gross appearance, clot sign, size, weight, volume and histology.

The subgroups below will be used for these analyses:

- *Stroke type (Ischemic or Hemorrhagic)*
- *Location of Intracerebral Hemorrhage (Deep or Lobar)*
- *Age (Less than 65 or 65 and older)*
- *Gender (Male or Female)*

- *Race (White, Black, Asian or Any other)*
- *Ethnicity (Hispanic or Non-Hispanic)*
- *Smoking status (Current, Former or Never smoked)*
- *Covid status (Active, Previous or No History of COVID)*
- *Time of stroke onset to procedure. (6 or less hours or More than 6hrs in Ischemic subgroup only)*

Descriptive statistics and 95% confidence intervals will be presented for each subgroup and the difference between subgroups. Notable differences between subgroups will be further evaluated using additional covariates and multivariate modeling to describe relationships between subgroups and clinical outcomes. These analyses will be performed in the Intent-to-Treat population given adequate numbers for robust analysis.

11. Pooling across centers

Analyses will be presented using data pooled across centers. Descriptive statistics for key variables such as age, baseline NIHSS, IV rtPA administration, stroke type, target vessel location, mTICI, and mRS will be presented to assess any potential site effects. If there is evidence of site heterogeneity, additional analyses will be performed to determine confounding factors influencing site and clinical outcomes. Appropriate stratified and modeling techniques, including contingency tables, logistic regression, and random effects will be used to assess differences between study centers to justify pooling data across centers. To avoid the influence of small enrolling sites on contingency tables, low enrolling sites will be removed as a sensitivity test for contingency table analyses.

12. Lost to follow-up and missing data

Every effort will be made to keep all missing data to a minimum. Patient discontinuation rates will be tabulated by the reason for early termination. Any

loss of viable or insufficient sample will be noted. The primary analysis will be data as observed.

13. Central Core and Pathophysiology Lab

In addition to the analysis detailed above, up to three independent laboratories will follow the Study Core Lab Charter to analyze collected samples via histology, proteomics, transcriptomics, and genomics.

14. Changes to planned analysis

All changes to the statistical analysis plan (SAP) will be documented in a revised SAP or in the clinical trial report.

15. References

1. Agresti (2014) Categorical Data Analysis. John Wiley & Sons: New Jersey

16. Revision History

Version	Description of Changes	Prepared by
1	Initial Release	
2	Revised to reflect increased enrollment of updated protocol. Clarified subgroup analysis categories. Added section 16 Revision History	
3	Revised Section 9: Analysis of adverse event (AE) section as we are collecting AE for the RED subgroup in Protocol E	