STATISTICAL ANALYSIS PLAN

CLP-21340

THUNDER: Acute Ischemic Stroke Study with the Penumbra System® including **Thunder**bolt™ Aspiration Tubing

Version 1.0

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1 Introduction

This statistical analysis plan (SAP) outlines details of the statistical analyses that have been described within the protocol and will describe analysis conventions. The SAP will be signed off prior to database lock.

2 Study Design Overview

This is a prospective, multicenter, single-arm study assessing the safety and efficacy of the Penumbra System including Thunderbolt™ Aspiration Tubing (Thunderbolt) in a patient population with acute ischemic stroke (AIS) secondary to intracranial large vessel occlusion (LVO) who are eligible for mechanical thrombectomy.

Up to 235 subjects will be enrolled from up to 35 centers. Each site will be limited to a maximum enrollment of 50 subjects (~20% of total enrollment).

The Primary Endpoint is as follows:

> Angiographic revascularization of the occluded target vessel at immediate post-procedure as defined by mTICI 2b or higher.

The Secondary Endpoints are as follows:

- Angiographic revascularization of the occluded target vessel after first pass as defined by mTICI 2b or higher.
- Angiographic revascularization of the occluded target vessel at immediate post-procedure as defined by mTICI 2c or higher.
- Time to revascularization.
- Modified Rankin Scale (mRS) 0-2 at 90 days
- Occurrence of symptomatic intracranial hemorrhages (sICH) at 24 hours.
- All-cause mortality at 90 days.
- Incidence of device related and/or procedure related Serious Adverse Events (SAEs) within 24 hours post-procedure.

3 Sample Size

The sample size calculation was based on the primary endpoint. All other endpoints will be secondary.

The targeted sample size for this study is 211 participants treated with Thunderbolt. To allow for approximately 9% attrition, enrollment will be up to 235 participants [211 evaluable participants/(1-0.09 attrition rate)=231.9, rounded up to 235].

The efficacy sample size estimate is based on an estimated incidence rate of 80% for the primary efficacy variable. This estimate is based on the mTICI 2b-3 Post-Penumbra System rate 77.5% (145/187) seen in the 3D study All Patients Intent-to-Treat (ITT) population. The efficacy performance goal for participants treated with Thunderbolt is 71.5%. A sample size of 211 participants has 80% power to detect a mTICI 2b-3 rate that is greater than the performance goal.

The details and justification of the sample size are described in the following sections.

3.1 Efficacy Power

The power for a one-group, one-sided, alpha =0.025 single proportion was calculated for a normal approximation to the binomial test using SAS v9.4 (SAS Institute, Cary, NC). The expected efficacy of the Penumbra System with Thunderbolt was based on data from the 3D study (6). The 3D study All Patients Intent-to-Treat population observed a post Penumbra System mTICI 2b-3 rate of 77.5% (145/187; Clopper- Pearson 95% confidence interval 70.9% to 83.3%). We expect the performance of the THUNDER study to be similar in efficacy.

The power was estimated by comparing the expected efficacy rate (80%) to the threshold of 71.5%. The parameters and output of the SAS proc power procedure are provided for efficacy (Table 1). The power of demonstrating an efficacy rate greater than 71.5% is approximately 80%.

Table 1. Efficacy Sample Size Parameters and Output

Alpha	0.025
Sides	1
Efficacy proportion	0.80
Null Proportion	0.715
Power	0.809
N	211
Total N (with 9% attrition, rounded up)	235

4 Interim Analysis

No interim analyses are planned for the study.

5 Endpoint Analysis

The primary endpoint analysis will be performed on a minimum of 211 participants who have received intervention and have a post procedure core lab adjudicated mTICI score.

6 Analysis Populations

6.1 Definitions

- **Intent-to-Treat (ITT):** The ITT sample includes all participants who are enrolled. Participants are considered enrolled once they or their representative have provided consent and all eligibility criteria have been confirmed whether or not Thunderbolt was used. Participants enrolled but that did not receive intervention with the Penumbra System including Thunderbolt will be followed out to 72 hours of enrollment or discharge (whichever comes first) for safety and will be summarized separately in the clinical report.
- **Modified intent-to-treat (mITT):** A modified Intent-To-Treat (mITT) sample is defined as a subset of the ITT sample. The mITT sample will include enrolled participants that met all eligibility including angiographic criteria with attempted use of the Penumbra System catheter including Thunderbolt (prepped for intervention) regardless of whether the device contacted the subject or not. This is the primary population for all efficacy and safety analysis.

- **Per-Protocol:** In addition to the defined mITT analysis sample, a per-protocol (PP) sample is defined as a subset of the ITT sample. The per-protocol sample will include all mITT subjects that received intervention and do not have major protocol deviations. Major deviations are those which affect the scientific soundness of the research plan or the rights, safety, or welfare of human subjects. These include, but are not limited to, missing primary endpoint data, informed consent omission or error, unauthorized device use, failure to report unanticipated adverse events to the IRB and/or FDA, and major eligibility violations. This is a secondary population for the primary efficacy outcome.

7 Patient Disposition

7.1 Definitions

The number of subjects for each of the following categories will be summarized.

- Total Screen Failures
- Enrolled
- Subjects completing the study; subjects not completing the study
- Subjects in the ITT population
- Subjects included in the mITT population
- Subjects included in the PP population

8 General Statistical Methods

Summary tables (descriptive statistics and/or frequency tables) will be provided for all baseline variables, efficacy endpoints, and safety endpoints as appropriate. Continuous variables will be summarized with descriptive statistics, such as n, mean, standard deviation, min, max. If a continuous variable has a highly skewed or strong non-normal distribution, the first, second (median), third quartile, and inter-quartile range will be used to summarize the data. Frequency counts and proportions will be provided for categorical data. All confidence intervals will be two-sided with alpha = 0.05, unless otherwise noted. Continuous variables will be presented with parametric 95% confidence intervals for the mean and categorical variables will be presented with Clopper-Pearson Exact confidence intervals.

Grouped analyses will be conducted using Fisher's exact test for categorical variables. Wilcoxon rank sum, t-test Kruskal-Wallis, or ANOVA will be used for univariate continuous analyses. Logistic, Cox, or

linear regression will be used for multivariable analyses as appropriate. Statistical tests and models will be applied as appropriate. All p-values presented will be two-sided, alpha=0.05 unless otherwise noted.

Analyses will be conducted using SAS© software Version 9.4 or higher (SAS Institute, Cary, NC).

9 Baseline Characteristics

Baseline data including, but not limited to demographic, clinical characteristics, and procedure data will be summarized using descriptive statistics.

10 Safety Analysis

10.1 Secondary Safety Endpoints

The secondary safety endpoints will be assessed with descriptive statistics and the associated 95% confidence interval. The additional secondary analyses include the following:

- Occurrence of symptomatic intracranial hemorrhages (sICH) at 24 hours.
- All-cause mortality at 90 days
- Incidence of device related and/or procedure related Serious Adverse Events (SAEs) within 24 hours post-procedure.

10.2 Analysis of Adverse Events

Adverse events will be coded using the MedDRA dictionary. The number and percentage of subjects with adverse events (AE) and serious adverse events (SAE) will be summarized by System Organ Class and preferred term. Each subject will be counted only once within a category by using the adverse event with the highest severity within each category. The CEC/DSMB adjudicated data supersedes the investigator reported data in analyses of adverse events.

An AE or SAE will be attributed as related to the device if it was classified as a definite or probable relationship. An AE or SAE will be attributed as related to the procedure if it was classified as a definite or probable relationship.

All information pertaining to adverse events noted during the study will be listed by subject, detailing verbatim given by the investigator, category, date of onset, date of resolution, causality and seriousness. The onset of adverse events will also be shown relative (in number of days) to the day of treatment.

All adverse events (AEs) will be analyzed with specific attention to the rate of all intracranial hemorrhage (ICH), including symptomatic intracranial hemorrhage (sICH), subarachnoid hemorrhage (SAH) and death.

The secondary safety endpoint will include the following device related and/or procedure related SAE within 24 hours post-procedure: symptomatic intracerebral intracranial hemorrhage (sICH), embolization to a new territory (ENT) and intracranial vascular injury (e.g. dissection or perforation).

11 Efficacy Analysis

11.1 Primary Efficacy Endpoint

The primary efficacy variable is proportion of patients with post procedure mTICI 2b or greater. If the Penumbra System was able to achieve a mTICI 2b or greater in the target vessel prior to use of any adjunctive devices (i.e. stenting, angioplasty, stent retriever, other non-Penumbra thrombectomy devices) then the procedure will be analyzed as a primary efficacy success. Subjects with intracranial stenosis/atherosclerosis (ICAD) treated with stenting or angioplasty at primary target vessel occlusion location prior to achieving mTICI 2b or greater will be analyzed as efficacy endpoint failures. The proportion of patients who were successful based on this criterion will be calculated along with the corresponding 95% Clopper-Pearson confidence interval. An exact binomial test will be used to test the null hypothesis that the efficacy rate is less than or equal to the acceptance criteria (71.5%) at a one-sided alpha = 0.025. This is equivalent to evaluating that the lower bound of the 95% confidence interval for the efficacy rate is above 71.5%.

$$H_0: P_{TICI} \leq 71.5\%$$

$$H_A: P_{TICI} > 71.5\%$$

Only Core Laboratory data will be used in the primary efficacy endpoint analysis of mTICI.

11.2 Secondary Efficacy Endpoints

The secondary endpoints will be assessed with descriptive statistics and the associated 95% confidence bound. The additional secondary analyses include the following:

- Angiographic revascularization of the occluded target vessel after first pass as defined by mTICI 2b or higher.
- Angiographic revascularization of the occluded target vessel at immediate post-procedure as defined by mTICI 2c or higher.
- Time to revascularization, defined as time from arterial puncture to mTICI 2b-3 first reached: Time of reported arterial puncture per investigator to time of first mTICI 2b-3 reached as seen on digital subtraction angiography per Core Lab review will be computed.
- Modified Rankin Scale (mRS) 0-2 at 90 days.

12 Subgroup Analysis

To evaluate the impact of baseline conditions on the primary endpoint, subgroup analyses will be performed for primary safety and primary efficacy variables. The subgroups below will be used for these analyses:

- **Age** (< 65, or >= 65 years)
- **Sex** (male, female)
- **Race/Ethnicity**
- **Late window onset to puncture (**=<6hrs, or > 6hrs**)**

Descriptive statistics and 95% Clopper-Pearson confidence intervals will be presented for each subgroup and the difference between subgroups. If a subgroup analysis shows notable differences between groups, additional analyses will be performed. Additional analyses will include covariate and multivariate modeling to describe relationships between subgroups and the endpoints to further evaluate homogeneity of the treatment effect.

Subgroup analyses for the efficacy endpoint and safety endpoint will be evaluated on the mITT population.

13 Handling of Multiplicity

There will be no adjustment for multiple comparisons to the primary endpoint.

The secondary safety endpoints will be presented as descriptive statistics with associated 95% Clopper-Pearson confidence intervals without multiplicity adjustment.

The secondary efficacy endpoints will be presented as descriptive statistics with associated simultaneous confidence intervals, adjusted using the Bonferroni method.

No adjustments for multiple comparisons/multiplicity will be made to subgroup confidence intervals or p-values.

14 Pooling Across Centers

The clinical study will be conducted under a common protocol for each investigational center with the intention of pooling the data for analysis. Every effort will be made to promote consistency in study execution at each investigational center. Analyses will be presented using data pooled across centers. In addition to the primary safety and efficacy endpoints, critical covariates (including, but not limited to age and sex) will be summarized by site. A Fisher's Exact statistical test will be performed for the - primary endpoint proportion to assess the heterogeneity of study results across sites. To avoid the influence that low enrolling sites may have on high enrolling sites, low enrolling sites will be removed from the initial Fisher's Exact test of heterogeneity. If there is evidence of site heterogeneity ($p<0.15$), additional analyses will be performed to determine confounding factors influencing site and primary endpoint. If required, the 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.

15 Blinding

The Penumbra Inc. clinical team responsible for the conduct of the study, the investigator, study center personnel, core laboratory and clinical events committee will not be blinded to each patient's treatment throughout the course of the study.

16 Lost to Follow-Up and Missing Data

For sensitivity purposes, additional analyses will be conducted for the primary efficacy endpoint. For those subjects without an evaluable mTICI score:

- Their primary efficacy outcome will be imputed using the worst clinical scenario assuming the subject experienced a primary efficacy mTICI 0-2a.
- Their primary efficacy outcome will be imputed using the best clinical scenario assuming the subject experienced a primary efficacy mTICI2b-3.

If needed, additional sensitivity analysis will be performed by multiple imputation. Critical baseline covariates will be used to predict the value of the missing endpoint data. Multiple iterations of the endpoint predictions will be performed (M≥2) and the multiple iterations will be pooled to obtain the final estimates of the primary efficacy endpoint (5).

For secondary outcomes and subgroup analyses, only subjects with complete data will be analyzed.

Patient discontinuation rates prior to the study completion will be tabulated by reason for early termination.

17 Committees

17.1 Clinical Events Committee (CEC)/ Data Safety and Monitoring Board (DSMB) Joint Committee

A CEC/DSMB will review and adjudicate safety endpoint AEs for seriousness, causality, and attribution. The CEC/DSMB data will supersede the Investigator-reported data in analyses.

17.2 Imaging Core Laboratory

The independent imaging core lab will review pre-procedure and procedural images to determine at minimum mTICI and 24-hour CT for ECASS defined ICH. The core lab data will supersede the Investigator-reported data in analyses of post procedure mTICI and ENT.

18 Changes to Planned Analyses

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

19 References

1. Agresti A. *Categorical Data Analysis*. John Wiley & Sons. Inc., Publication. 2002.
2. Higgins, James J. *Introduction to Modern Nonparametric Statistics.* Brooks/Cole—Thomson Learning, Inc. 2004.
3. SAS Institute Inc. 2023. *SAS/STAT®9.4 User's Guide, Second Edition*. Cary, NC: SAS Institute.
4. *ICH: E9 Statistical Principles for Clinical Trials.* European Medicines Agency. 1998.
5. Yan X, Lee S, Li N. Missing data handling methods in medical device clinical trials. J Biopharm Stat. 2009;19(6):1085-1098. doi:10.1080/10543400903243009.
6. Nogueira, R. G., Frei, D., Kirmani, J. F., Zaidat, O., Lopes, D., Turk, A. S., III, . . . for the Penumbra Separator, D. I. (2018). Safety and Efficacy of a 3-Dimensional Stent Retriever With Aspiration-Based Thrombectomy vs Aspiration-Based Thrombectomy Alone in Acute Ischemic Stroke Intervention: A Randomized Clinical Trial. JAMA Neurology, 75(3), 304-311. doi:10.1001/jamaneurol.2017.3967

20 SAS Code and Output

20.1 Primary Efficacy Power Calculation

```
proc power;
        onesamplefreq
        test = Z
        method=normal
        alpha = 0.025
        proportion = 0.80
        nullproportion = 0.715
        ntotal = 211
        power = .
        sides=U;
run;
```

The POWER Procedure
Z Test for Binomial Proportion

Fixed Scenario Elements	
Method	Normal approximation
Number of Sides	U
Null Proportion	0.715
Alpha	0.025
Binomial Proportion	0.80
Total Sample Size	211
Variance Estimate	Null Variance

Computed Power
0.809