STATISTICAL ANALYSIS PLAN

STRIDE: A **S**tudy of Patients with Lower Extremity Acu**t**e Limb Ischemia to **R**emove Thrombus with the **I**n**d**igo® Aspiration Syst**e**m

Protocol

CLP-15549

Version

26-Jan-2023

Contents

1 Overview

This is a 12-month post-market, real world, prospective, single-arm, multi-center study to collect safety and performance data on the Indigo Aspiration System in a patient population with lower extremity acute limb ischemia (LE ALI).

Up to 130 patients presenting with LE ALI who are eligible for mechanical thrombectomy using the Indigo Aspiration System and who meet the inclusion, exclusion criteria will be enrolled at approximately 25 sites globally. Each site will be limited to a maximum enrollment of up to 26 patients (~20% of total enrollment).

Subjects will be in the study for approximately 12 months from enrollment to last follow-up. Follow-up assessments will occur at discharge and/or 7 Days whichever occurs first, 30 Day (± 14days), 180 Day (± 30days), and 365 Day (± 60days) following the procedure. Every follow-up treatment of patients will be recorded for the entire duration of the study. It is anticipated that enrollment will take approximately 24 months.

2 Study Objectives

The primary objective of interest is the target limb salvage rate at 30 Day post-procedure. The study limb salvage rate at 1-month (30 Day) post-procedure will be compared with the combined historical control rate of 95.7% observed under current standards of care.

The secondary objective of the study includes secondary performance and safety endpoints as detailed in the statistical analysis section below.

3 Sample Size

The sample size is calculated under the assumption of non-inferiority of the Indigo aspiration system in patients with LE ALI compared to current standards of care. The observed combined historical control rate of limb salvage is 95.7% under current standards of care (Appendix A). We assume that 94% (108/115) of the study subjects will achieve target limb salvage 1-month (30 Day) post-procedure.

Based on a binomial analysis with a non-inferiority margin of 10%, a study of 115 enrolled subjects will have approximately 80% power with a one-sided alpha of 0.025. The sample size was adjusted to 130 subjects to account for approximately 10% attrition.

Table 1:

Sample size calculation for Clinical Study with INDIGO Aspiration System in Acute Limb Ischemia CLP-15549.

Method	Normal Approximation
Number of Sides	Upper one-sided with alternative greater than null value
Null Proportion	0.957
Margin	-0.1
Alpha	0.025
Binomial Proportion	0.94
Sample Size	115
Variance Estimate	Null Variance
Computed Power	0.805

4 Interim Analysis

No interim analyses are planned for the purpose of terminating the study for a positive result. No adjustments will be made to the confidence bounds for the final analysis. Interim analysis may be performed for purpose of publication of study results.

5 General Statistical methods

Baseline data including, but not limited to demographics, clinical characteristics, and angiographic characteristics will be summarized using descriptive statistics. For categorical outcomes, the 95% two-sided confidence intervals will be calculated. For continuous outcomes, a 95% confidence bound for the mean or mean change from pre-procedure will be constructed. Results collected at multiple visits will be summarized at each visit, as necessary. Summaries for all measures will include all observed data for each visit. All analysis will be performed on the intent-to-treat population.

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

6 Patient Disposition

The number of subjects for each of the following categories will be summarized for final analysis:

6.1 Screened

Screened subjects are all subjects considered for participation in the study, whether or not they sign informed consent.

6.2 Screen Failure

Screen failure subjects are all subjects considered for participation in the study, who failed to meet inclusion criteria or met exclusion criteria. Patients can be screen failed based on general criteria. These patients may or may not have signed an informed consent form.

6.3 Enrolled

An eligible patient is considered enrolled once informed consent is obtained and the Indigo Aspiration Catheter has been inserted into the patient's body.

6.4 Completed

Completed subjects are all subjects who were enrolled and completed the study follow-up or were known to have died prior to the last follow-up timepoint. The completed subject metric will be provided for 365 Day follow-up.

6.5 Early Termination

Early termination subjects are all subjects who were enrolled but did not complete follow-up and were not known to have died. The early termination subject metric will be provided for 365 Day follow-up.

7 Definition of Analysis Population

All performance and safety outcome measures will be analyzed under the intent-to-treat (ITT) principle. Under this principle, the ITT sample includes all subjects who are enrolled. This population is the primary analysis population.

A per-protocol (PP) sample is defined as a subset of the ITT sample. The per-protocol sample will include all subjects that do not have major protocol deviations (eg. eligibility violation or informed consent violations). In addition to the ITT, a per protocol analysis will be done for the primary and secondary endpoints.

8 Statistical Analysis

8.1 Primary Endpoint Analysis

The primary endpoint is a safety endpoint consisting of the target limb salvage rate at 1-month (30 Day) post-procedure. Limb is considered salvaged if no amputation was performed on target limb within 30 Day post procedure, or if target limb was amputated at or distal to the tarsal/metatarsal joint. The primary safety analysis will be the difference between the study group and the combined historical control rate of 95.7% observed under current standards of care.

The null hypothesis is that the difference between the primary endpoint rate and the standard of care at 30 Day post-procedure is less than or equal to -10%. The alternative hypothesis is the difference at 30 Day post-procedure is greater than -10%. Formally, the null and alternative hypotheses to be tested are as follows:

$$H_0: P_{study} - 0.957 \leq -0.10$$

$$H_A: P_{study} - 0.957 > -0.10$$

Where P_{study} is the proportion of patients who achieve target limb salvage 30 Day post-procedure.

The primary safety endpoint is met if the lower limit of the two-sided 95% normal approximated confidence interval of the primary endpoint rate is greater than 85.7%. The primary safety analysis will be unadjusted.

8.2 Secondary Safety and Efficacy Endpoint Analysis

The secondary performance and safety endpoints will be assessed with proportions based on the endpoint criteria and accompanying 95% confidence intervals presented. The secondary endpoints are as detailed below:

- Technical success defined as TIMI 2 or TIMI 3 flow rate immediate post-procedure. If performed, the TIMI score after adjunctive procedure will be considered as immediate post-procedure
- Modified SVS runoff score immediate post-procedure as compared to baseline (pre-procedure score). If performed, the modified SVS score after the adjunctive procedure will be compared to pre-procedure. The modified SVS runoff score will be analyzed as a continuous outcome, with the paired difference being compared to 0 (no difference). The modified SVS runoff score will also be evaluated as a categorical variable as an exploratory analysis.
- Improvement of Rutherford classification of one or more unit at discharge as compared to pre-procedure. The number and percent of subjects in each Rutherford category at baseline and at discharge will be reported.
- Patency at 1-month (30 Day): Patency is defined as a target lesion without a hemodynamically significant stenosis/re-occlusion on duplex ultrasound (>50%,) and without target lesion reintervention (TLR). Patency failure of the target vessel occurs when clinically significant stenosis/re-occlusion requires reintervention.
- Target limb salvage rate at 12 months (365 Day) post-procedure.
- Rate of device related serious adverse events (SAEs).
- Major bleeding peri-procedure (on the day of and/or the day after the procedure) defined as fatal or leading to a drop in hemoglobin of ≥ 5 g/dl, or significant hypotension with the need for inotropes, or requiring surgery (other than vascular site repair), or symptomatic intracranial hemorrhage (ICH), or requiring transfusion of two or three units of red blood cells or equivalent whole blood.
- Mortality rate at 12 months (365 Day) post-procedure.

8.4 Handling of Multiplicity

There will be no adjustment for multiple comparisons on the primary effectiveness variable since there is only one primary endpoint.

8.5 Analysis of Deaths

The Kaplan-Meier product-limit method will be utilized to assess the mortality rate. With the date of procedure set at Day 0, any death occurring on or before day 365 will be counted as a death. Patients who are alive past 365 days will be censored at 365 days. If clinical assessment is missing for a patient who has not died, the patient will be censored at the last follow-up date. The time to death will be plotted with confidence intervals.

Additionally, a cross-sectional analysis of mortality at 365 days will performed. The frequency and proportion of deaths will be presented with the 95% confidence interval.

8.6 Analysis of Adverse Events

All adverse events, including serious adverse events (SAE), adverse device effect (ADE), and unanticipated adverse device effect (UADE) will be summarized by showing the number and percent of subjects which report the event. Events will also be reported by relationship to the procedure or device. Adverse events judged as probably or definitely related to the Indigo Aspiration System or procedure will be analyzed as device related or procedure related respectively.

Adverse events will be coded using the MedDRA dictionary. The number and percentage of subjects with AEs and SAEs will be summarized by body system and preferred term. Each subject will be counted only once within a category. An overall listing of each category will be provided.

9 Subgroup Analysis

To evaluate the association of baseline condition on outcomes, subgroup analyses will be performed for the primary efficacy variable (Target limb salvage at 30 Days), select secondary endpoints, patency at 30 Day (defined previously), and mortality at the 365 Day. The subgroups below will be assessed provided there are adequate sample size for analysis. Descriptive statistics will be presented for each subgroup with a 95% confidence interval for the difference between two groups.

- Thrombus and Lesion Location. The lesion location is the location of the underlying atherosclerotic disease.
    - Subjects with at least one thrombus located in the femoral-popliteal region (or proximal) vs subjects with tibial or distal vessel thrombus.
    - Subjects with at least one lesion located in the femoral-popliteal region (or proximal) vs subjects with tibial or distal vessel lesion.
- Severity of ALI at baseline (Rutherford classification IIA vs IIB)
- History of diabetes (Yes vs No)
- Index procedure in graft (Yes vs No)
- History of chronic limb ischemia (Yes vs No)

10 Pooling Across Centers

To assess the validity of data pooling, heterogeneity across sites and regions (North America vs Europe) will be examined using relevant methods, such as random effect models, ANOVA (continuous variables), contingency tables or binary logistic regression (categorical variables), GLM or Cox proportional hazards (event time variables) as appropriate. 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.

11 Lost to Follow-up and Missing Data

Every effort will be made to minimize missing data.

For sensitivity purposes, the following additional analyses will be conducted if data for the primary endpoint, limb salvage at 30 day, is missing:

- Analyze only subjects with complete 30 Day data.
- Subjects that were lost to follow-up before 30 days will be imputed as limb lost for the primary outcome.
- Subjects that were lost to follow-up before 30 days will be imputed as limb salvaged for the primary outcome.

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

12 Imaging Core Lab

The independent imaging core lab will review images from the pre-procedure and post-procedure (pseudonymized) angiograms to determine at minimum, the TIMI scores. An imaging core lab charter will provide the procedure for the core lab review.

13 Changes to Planned Analysis

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

14 References

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ischemia: 30-day results from a single-center experience. Catheter Cardiovasc Interv. 2012;80(4):636-43.

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15 Revision History

Version	Prepared by	Description of changes
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
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Appendix A – Justification for Sample Size Calculation

The table below provides the literature search results for publications of limb salvage rate in acute limb ischemia patients 30 days after endovascular treatment. This supports a standard of rate primary endpoint rate of 95.7%.

PMID/DOI (Lead Author)	**Limb Salvage Rate at 30 day**
30043994 (Grip et al)	6298/6493
18726955 (Ansel et al)	54/57
11287526 (Kasirajan et al)	76/86
28555191 (Heller et al)	144/147
28458517 (Kronlage et al)	196/202
20153667 (Kropman et al)	769/895
22511320 (Gupta et al)	24/24
DOI: 10.3969/j.issn.1008-794X.2017.06.008 (Gong et al)	11/12
20044798 (Oğuzkurt et al)	29/29
24360240 (Byrne et al)	142/147
Total:	7743/8092
Rate:	0.957