CLOT OUT STUDY

Clotild® Smart Guidewire System evaluation in Endovascular Thrombectomy procedure

-

STATISTICAL ANALYSIS PLAN (SAP)

Sponsor:

Sensome SAS
Immeuble Odyssée
2-12 rue du Chemin des Femmes
91300 Massy
FRANCE

Version 2

Date: 13/11/2023

SIGNATURES:

This Statistical Analysis Plan has been approved by the Statistician and the Sponsor. The following signatures document this approval.

Tine Willems, PhD, Biostatistician, T!Write	Date
Sponsor – Sensome represented by Joachim Rambeau, Applied research scientist	Date
Sponsor – Sensome represented by Julie Lafaurie, Clinical and Preclinical Lead	Date

Table of contents

Change history

Version	Date	Specification of Change
1	25/08/2022	Initial Release
2	13/11/2023	Update to align to CIP V6: - Addition of 2 secondary endpoints and 6 tertiary endpoints - Revision of sample size calculation and statistical methodology from machine learning to more empirical methods

1. Introduction

The purpose of this Statistical Analysis Plan (SAP) is to prospectively outline the types of analyses and presentations of the data that will form the basis for conclusions regarding this clinical investigation. The analyses defined in this plan should answer the safety and performance objectives outlined in the Clinical Investigation Plan (hereunder called CIP or protocol), and explain in detail how the data will be handled and analysed, adhering to commonly accepted standards and practices for biostatistical analysis in the medical device industry.

This document contains information to support the generation of a Clinical Study Report (CSR) for Clinical Investigation Plan SEN_CLOTILD_FIH_1, including detailed descriptions of the statistical methods to be applied, as well as the analysis summary tables and figures and patient data listings intended to present the analysis results. The analyses described are based on the final CIP (version 6, 12 June 2023). The SAP will be finalized prior to database lock and describes the statistical analysis as it is foreseen when the study is being planned. If circumstances should arise during the study rendering this analysis inappropriate, or if improved methods of analysis should arise, updates to the analyses may be made. Any deviations from the SAP after database lock, reasons for such deviations and all alternative or additional statistical analyses that may be performed, will be described in a SAP Addendum.

The planned analyses identified in this SAP may be included in regulatory submissions, medical presentations and manuscripts. Exploratory analyses, not identified in this SAP, may be performed to support the clinical development program. Any post-hoc or unplanned analyses that are performed but not identified in this SAP will be clearly identified in the CSR.

2. Study objective

The objective of the study is to evaluate the safety and the possibility of Clotild® Smart Guidewire System (CSGS) to provide electrophysiological measurements. The electrophysiological measurements will be used to update CSGS's database and thus improve the prediction accuracy of the model in providing physicians with insights for mechanical thrombectomy.

The **primary objective** of the study is to evaluate the **safety** of using Clotild® Smart Guidewire System (CSGS) at the occlusion location during an EndoVascular Thrombectomy (EVT) procedure for the treatment of subjects with acute ischemic stroke eligible for EVT.

Safety will be evaluated by means of the amount of intracranial vessel perforations and/or dissections with the CSGS during the procedure.

The **secondary objective** of the study is to evaluate the **performance** of CSGS, defined here as the feasibility to measure electrophysiological properties of the occlusion in vivo during EVT procedures for the treatment of acute ischemic stroke.

Performance will be evaluated by the ability to correctly binary classify the clot as Red Blood Cell (RBC) positive or RBC negative, platelet positive or negative and fibrin positive or negative. Performance endpoints that focus on clot characterization are split in two categories: one category that demonstrates the ability of an automatic system to correctly identify the local composition of a clot

(local-scale), and the other category that reveals the extent of concordance between a number of local scale measurements made from the CSGS and the histological characterization by the Core Laboratory (Core Lab) of the full clot retrieved during the EVT procedure (clot scale).

The **tertiary objective** of the study is to explore possible relationships between CSGS measurements and intervention parameters such as first pass effect, number of passes to successfully retrieve the clot and other key parameters. Due to the nature of the tertiary objectives, not all centers may contribute to the data collection.

3. Study design and plan

This study is a prospective multi-centre, single-arm study to evaluate the safety and the performance of Clotild® Smart Guidewire System during endovascular thrombectomy (EVT) procedures, in subjects presenting acute ischemic stroke. The study will be performed in Australia and Europe.

Since this is a single-arm study, no control treatment is determined and no randomisation will occur. Subjects and investigators are not blinded for the treatment. Study data are captured until 24 hours (range 16-36 hrs) after the procedure. Assessment of the safety endpoint will be performed by an independent core imaging laboratory. Evaluation of the performance endpoints will be done by comparison of the predictions made by phenomenological modelling techniques with expert annotations of the sensor measurements and the histological evaluations by an independent core histology laboratory.

An overview of all follow-up times and respective captured information can be consulted in **table 1.**

The objectives and endpoints will be based on the data collected during the procedure and short follow-up period. There is a primary safety endpoint and a primary performance endpoint. There are 7 secondary endpoints and 6 tertiary endpoints that will be evaluated.

Table 1. Study activity overview

Parameter/Examination	Screening/Baseline	EVT procedure	24H (range 16- 36 hrs.) Post-procedure
Inclusion/Exclusion criteria	X		
Demographics & Medical History incl. Time of stroke onset, NIHSS, GCS	X		
Pregnancy test**	X		
Vital Signs (inc. ECG at screening)	X	X	
Laboratory assessments	X		
Patient Information/ informed consent	X		

Neuro Imaging exams	CT/CTA / MRI/MRA	DSA CT/CTA/MRI/MRA*	CT/CTA/MRI/MRA***
Timings (imaging, tPA, arterial puncture, recanalization)		X	
Thrombus collection		X	
AE/SAE		X	X
Concomitant medication, incl. Anti-thrombotic treatments	X	X	X

* if available per local hospital practice

** according to site specific standard of care (e.g. test, verbal communication)

*** CT/CTA / MRI/MRA if available per local hospital practice (range 2hrs - 36hs post procedure)

4. Endpoints

a) Primary Safety Endpoint

The Primary Safety Endpoint is defined as the proportion of patients having intracranial vessel perforation and / or dissection due to CSGS usage at the site of usage in intracranial vessels by assessment by Interventional Neuroradiologist during the procedure and final adjudication of the DSA (Angiogram) by the Data Safety Monitoring Board.

The Interventional Neuroradiologist will complete if a vessel perforation/dissection due to CSGS usage at the site of usage occurred in the eCRF. The imaging Core Lab will assess the procedural images and will independently assess if a vessel perforation/dissection due to CSGS usage at the site of usage occurred. If one of both (investigator of Core Lab) suspects a vessel perforation/dissection, the DSMB will adjudicate if or not a vessel perforation/dissection occurred.

The proportion will be determined by

$$\frac{number\ of\ patients\ with\ perforation\ or\ dissection}{number\ of\ patients\ in\ which\ the\ guidewire\ went\ through\ the\ sheath}$$

Furthermore, a 95% confidence interval will be estimated.

b) Primary Performance Endpoint

The Primary Performance Endpoint is defined as the ability to perform binary classification of individual electrophysiological parameter measurements by distinguishing local regions with substantial red blood cell content (RBC-positive) from regions with negligible red blood cell content (RBC-negative) in the occlusion. This endpoint can be understood as the ability for an automated system to rank individual electrophysiological measurements in the occlusion according to the local scale content of RBC (the predicted RBC-content score).

The ability to perform binary classification will be evaluated by the sensitivity (true positive rate) and the specificity (false positive rate) for varying detection threshold. Based on those 2 metrics, the Area under the Receiver Operator Characteristic curve (AUC of ROC) and its 95% confidence intervals (CI)

will be calculated. An AUC of 0.85 is assumed computed from the RBC-content score predicted on the validation dataset only with a maximal width of 0.1 for the 95% confidence interval.

The 'gold standard' is defined as the binary classification of the local regions into RBC-positive or RBC-negative content performed by the Sensome experts. This labelling, considered as ground truth, is made by (at least) two independent SENSOME experts that cumulate the relevant experience in electrochemical impedance measurements of different biological tissues from previous non-clinical research. The comparator will be the binary classification determined by the CSGS model developed by phenomenological modelling. The model will be developed on the data of the development dataset while it will be validated on the data of the validation dataset.

The single electrode pair measurement is defined as the response to an applied micro-current through an electrode pair. Such a response is referred to as `the electrophysiological parameter': it depends on the arrangement and type of cells and interspersed biochemical components. The response across a single electrode pair is composed of a set of real numbers (up to 32 for CSGS) which are not independent; this set of numbers is referred to as a `spectrum' since it forms the response as the micro-current frequency is swept across a pre-defined range. This is also referred to as a `local-scale measurement'. A measurement triggered by the Cloviz software collects at a given time point the spectrums from a pre-defined set of electrode pairs. This is also referred to as a `sensor-scale measurement' since it sweeps the electrode pairs of the sensor.
The CSGS will be inserted and up to 6 measurements will be done: 1 measurement distal to the access catheter in circulating blood (this is the reference measurement), 1 measurement proximal to the site of occlusion, 2 to 3 measurements inside the occlusion and 1 measurement at the most distal advancement of the guidewire (ideally distal to the occlusion). The collection of measurements made in contact with the clot is referred to as the `clot-scale' measurement.

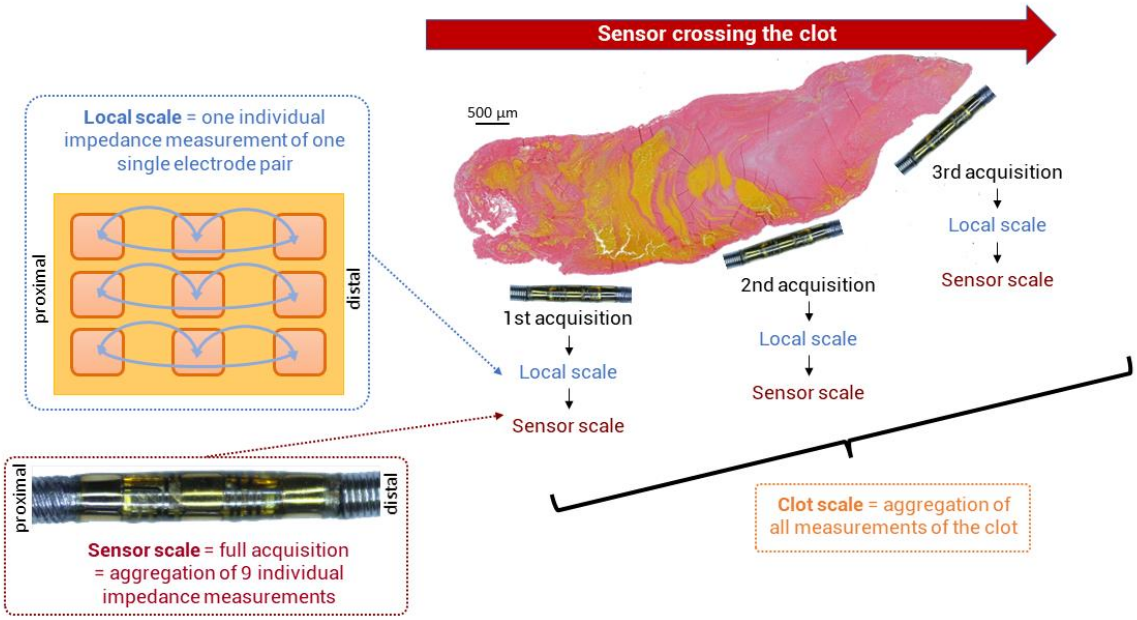

Figure 1: Schematic view of the sensor presenting the different scales of measurements.

Data from the CSGS is synced to the cloud database. These data are processed offline. Individual electrophysiological measurements are annotated manually by Sensome experts by inspecting the signal and the histological content from the full clot.

c) Secondary Endpoints

There are 7 secondary endpoints. The secondary endpoints are all performance endpoints and include:

1. The concordance between occlusion measurement done by CSGS, and the histopathology results of the clot retrieved during the EVT procedure, at the level of red blood cell content in the occlusion (clot scale).
   To determine the histopathological results of the clot, the mean will be taken of the percentage RBC composition of the 3 analyzed slices (value between 0 and 1) provided by the Core Lab.
   Significant correlation will be judged at the 0.05 significance level by the t-statistic of the relation between RBC content as output by the prediction model and the RBC content as quantified by the MSB-staining histological analysis
2. The ability of CSGS detecting the proximal end of the occlusion (sensor-scale), as compared to the physician's labelling (tag 'PRE-CLOT' for no occlusion contact) and tag 'CLOT' for occlusion contact). The concordance will be judged by the Area Under the Receiver Operator Characteristic curve on the validation set. The same methodology will be used as for the primary performance endpoint. Data from the interventions in the development and validation sets will be the same as for the primary endpoint.
3. Procedural success defined as the ability to navigate CSGS to the occlusion and measure electrophysiological properties of the occlusion. Procedural success is achieved from the moment that at least 1 effective measurement is captured by the CSGS in or beyond the clot.
4. The ability to perform binary classification of individual electrophysiological parameter measurements (local scale) by distinguishing local regions with substantial platelet content (platelet-positive) from regions with negligible platelet content (platelet-negative) in the occlusion. The ability to perform binary classification will be evaluated by the performance metric area under the ROC curve computed from the platelet-content score predicted on the validation set. The same methodology will be used as for the primary performance endpoint.
5. The concordance between aggregated occlusion measurements (clot scale) done by CSGS, and the histopathology results of the clot retrieved during the EVT procedure, regarding platelet content in the occlusion.
   To determine the histopathological results of the clot, the platelet composition (value between 0 and 1) will be averaged over the 3 analyzed slides provided by the Core Laboratory.
   Significant correlation will be judged at the 0.05 significance level by the t-statistic of the relation between platelet content as output by the prediction model and the platelet as quantified by the CD42b immunochemistry staining histological analysis.
6. The ability to perform binary classification of individual electrophysiological parameter measurements (local scale) by distinguishing local regions with substantial fibrin content (fibrin-positive) from regions with negligible fibrin content (fibrin-negative) in the occlusion. The ability to perform binary classification will be evaluated by the performance metric area under the ROC curve computed from the fibrin-content score predicted on the validation set. The same methodology will be used as for the primary performance endpoint.
7. The concordance between aggregated occlusion measurements (clot scale) done by CSGS, and the histopathology results of the clot retrieved during the EVT procedure, regarding fibrin content in the occlusion.
   To determine the histopathological results of the clot, the fibrin composition (value between 0 and 1) will be averaged over the 3 analyzed slides provided by the Core Laboratory.

Significant correlation will be judged at the 0.05 significance level by the t-statistic of the relation between fibrin content as output by the prediction model and the fibrin content as quantified by the MSB-staining histological analysis.

d) Tertiary Endpoints

There are 6 tertiary more exploratory endpoints. The tertiary endpoints assess the correlation between the CSGS measurements with intervention parameters such as:

1. Arterial wall detection for individual measurement - The local detection of arterial wall vs. clot when clot-contact is ensured. Differentiation of the arterial wall cannot be validated by external means. Expert labelling will be done on tagged acquisitions (PRE CLOT, CLOT, CLOT or beyond, MOST DISTAL): for each electrode pair the expert will evaluate whether it is in contact with arterial wall or not. Comparison with the prediction model will be made at such local-scale (single electrode pair) by using the same performance metric as for the primary endpoint, i.e. by means of the AUC of ROC.
2. The ability of CSGS detecting the distal end of the occlusion. Two comparisons will be made: one with expert labelling on tagged acquisitions and one compared to external evaluation when distal injection of contrast agent allowed the core lab to evaluate the positioning. The expert will label the CLOT, CLOT or beyond, and MOST DISTAL tagged acquisitions as whether the sensor is in clot contact or not. The last clot contact will be used as the positive example and the first labelled non-contact measurement will be used as the negative example. Regarding the subset of interventions for which a distal injection of contrast agent allowed the identification of the location of the sensor in or behind the clot by the imaging core lab, the ground truth will be used identically on the last tagged acquisition in the clot and the first tagged acquisition where the sensor is established behind the clot. The AUC of ROC will be used identically that for the Secondary endpoint for the proximal detection. Data from the interventions in the development and validation sets will be the same as for the primary endpoint.
3. The number of passes - The prediction of the number of passes to remove the clot. Interventions with successful clot retrieval are used. The input may include all the electrophysiological measurements of the intervention. The output is the number of passes needed to remove the clot as reported by the investigator. Data from the interventions in the development and validation sets will be the same as for the primary endpoint.
4. The prediction of first pass effect. Interventions can be split into two groups whether or not a single pass was enough to achieve a TICI score better than 2b; this forms the positive and negative groups respectively. The prediction can be evaluated with a ROC curve and the AUC metric, as described for the Primary endpoint, but where the prediction is made at the intervention level. The same methodology will be used as for the primary performance endpoint. Data from the interventions in the development and validation sets will be the same as for the primary endpoint.
5. The prediction of the etiology of the clot. Data from the interventions in the development and validation sets will be the same as for the primary endpoint.
6. The prediction of the type of device that has better chances to remove the clot. Data from the interventions in the development and validation sets will be the same as for the primary endpoint.

5. Study Population

a) Treatments and subject enrolments

This study will be conducted in subjects presenting an acute ischemic stroke due to M1 occlusion, eligible for EVT based on neuro-interventionist and/or neurologist investigators' opinion.

Point of enrollment: "Patients are considered as enrolled only once the patient or if the patient is incapable of providing consent, once the patient's legally authorized representative has signed and dated the patient informed consent form as part of the informed consent process or confirmation of study participation by the individuals."

Subjects will undergo the EVT procedure in accordance with standard of care. CSGS will be inserted and up to 6 measurements will be done: 1 measurement distal to the access catheter in circulating blood (this is the reference measurement), 1 measurement proximal to the site of occlusion, 2 to 3 measurements inside the occlusion and 1 measurement at the most distal advancement of the guidewire (ideally distal to the occlusion). Each of these measurements will be tagged using the tablet interface.

The occlusion material recovered during the EVT will be immediately stored in a preservative solution of formaldehyde and sent to the histopathology lab for preparation. Analysis of histopathology of the occlusion will be conducted in a core central laboratory.

b) Inclusion and exclusion criteria

Inclusion criteria

Candidates for the study must meet ALL the following inclusion criteria:

1. Age > 18 years
2. Clinical signs and symptoms consistent with the diagnosis of an acute ischemic stroke eligible for EVT based on neurointerventionist and/or neurologist investigators' opinion.
3. Occlusion with origin in M1 on CTA or MRA of an intracranial vessel amenable to EVT. Patients with migrated clots outside M1 will not be treated with the study device.
4. Written Informed Consent to participate in the study. If the subject is incapable of providing consent due to the patient's condition and the urgency of the EVT procedure, the Informed Consent Form will be obtained as per local country practice and approved by the Ethics Committee and Regulatory Agencies (if appl.)

Exclusion criteria

Candidates for this study will be excluded if ANY of the following conditions are present:

1. Patient has an intracranial occlusion that does not originate in M1 or/and tandem occlusions
2. Current participation in another investigational device or drug study that has not completed the primary endpoint or that clinically interferes with the current study endpoints.
3. Candidates not eligible for EVT based on neurointerventionist and/or neurologist investigators' opinion.

4. Known lactating or confirmation of positive pregnancy test according to site specific standard of care (e.g. test, verbal communication).

6. Statistical basis for sample size

The sample size calculation is driven by the amount of patients needed in the development and validation dataset. The size of the validation set is justified by the minimal number needed to achieve a sufficiently precise estimate of the primary endpoint, the precision being computed as the width of the confidence interval for a given confidence level.

The sample size calculation is split in two parts, one for the development set and one for the validation set.

Development set size

The development set size was fixed to obtain a near-optimal RBC categorization performance (as defined in the primary performance endpoint), based on preliminary research performed by SENSOME on an ex-vivo dataset (DND_tr-0033-clot-composition-phenomenological-model-development_v1). The standard approach at Sensome was to use machine learning techniques to cope with the complexity of the signal. However, our accumulated experience guided the design of a phenomenological model (based on observations and on physical properties of the signal). Its performance on local-scale composition prediction, evaluated on the ex-vivo dataset, is comparable to the machine learning model performance previously developed. The fine-tuning of its parameters has got only a minor influence on the performance, as assessed on the ex-vivo dataset.

Its extrapolation for thrombectomy-like procedures requires an additional rule to distinguish the arterial wall and potentially a fine-tuning of handful of its parameters. Overall, the number of examples needed to complete the first local-scale clot composition prediction model is constrained by the observation of ideal cases (locally pure RBC content and locally pure platelets content). Based on the fraction of pure cases that was observed on the ex-vivo data set, and on rules to sub-sample this data set regarding the number of clot contact measurements per intervention, we find that N = 11 is an adequate estimate of the development sample size.

We thus require a number of $\mathbf{N_D = 11}$ interventions with at least 1 evaluable measurement in contact with the clot to be included in the development set to reach near optimal performance with respect to primary performance endpoint assessment (Development Performance Population).

Validation set size

The minimum number of N_V interventions to be included in the validation dataset to estimate the performance Â with sufficient statistical confidence margin is determined, with Â the area under the ROC curve (AUC) of the prediction model obtained on the validation sample of the population.

The following assumptions are made:

- **Estimated average number of independent individual measurements from a single tagged acquisition with clot contact = 5.44** (based on the quality check of data collected in the first 9 procedural successes)

- **Estimated average number of tagged acquisitions with clot contact per intervention = 3** (based on the quality check of data collected in the first 9 procedural successes)
- **Estimated number of RBC-positive cases = 53%** (based on the quality check of data collected in the first 9 procedural successes)
- **Expected number of RBC-negative cases = 47%**
- **An AUC of 0.85 is assumed with a maximal width of 0.1 for the 95% confidence interval**

With these assumptions, using the Hanley and McNeil estimate (Hanley and McNeil 1982) for the confidence interval to reach an expected AUC of 0.85 and a maximal width of the 95% confidence interval of 0.1, a number of **N_V = 14 interventions** with at least 1 evaluable measurement valid interventions are necessary for the validation set (Local-Scale Performance Population).

Total sample size

The total sample size has to be corrected for a 40% anomaly rate (see vi).

The **total sample size** N = (N_D + N_V)/0.60 = **42**.

7. Bias

Since this is a single-arm study, no randomization is performed. Investigator, nor patients are blinded for the treatment.

To minimize bias, an independent Data Safety Monitoring Board (DSMB), also known as a Data Monitoring Committee (DMC), will be responsible for monitoring safety and performance aspects of the study. Rules of operation and responsibilities will be outlined in the DSMB Charter.

The DSMB consist out of an uneven number of experts in the field, enabling the assessment and conclusion with majority votes. If needed, the DSMB may invite ad hoc team members, such as statistical support. These ad-hoc members will refrain of voting. To review safety events happening in the study – for some events, the DSMB may be requesting additional information from the investigational site or the Core Lab to allow a comprehensive review. DSMB will be responsible of adjudicating the primary safety endpoint.

Secondly, to evaluate the Primary Performance Endpoint a binary classification of individual electrophysiological parameter measurements into `RBC-positive' and `RBC-negative' is performed by 2 Sensome experts independently. In case of discrepancies, a third expert will decide on the classification. This process cannot be performed by independent reviewers. Experts involved in annotating the development set shall not inspect the incoming validation dataset before the development is declared finished (the prediction models being fixed). As dataset inspection is also routine to detect anomalies, another Sensome expert not involved in the development could be designated in the transition period (when the development phase is not terminated but the development set is completed).

Thirdly, an expert in the field of histopathology from an independent Core Lab (Mayo Clinic) will perform the histopathological analysis of the clots. The Core Lab will be blinded for the results of the CSGS. Rules of operation and responsibilities are outlined in the Core Lab Instructions.

Fourthly, another independent imaging Core Lab (UCLA) will check the images. The imaging Core Lab will be set-up by 2 experienced readers. Both will check independently the pre-procedural, procedural and post-procedural images. Both readers will assess the primary safety endpoint. In case of disagreement between the 2 readers, a decision will be made by consensus. Rules of operation and responsibilities are outlined in the Core Lab Instructions.

It is well known that some clots might not be available for histological assessment. Next to that, data captured by the CSGS can be anomalous/missing. So the measures of agreement will only be based on those clots or on data from the CSGS that are available. The proportion of available clots is estimated to be approximately 60% of the total procedures. The proportion of available CSGS data is estimated to be 60% (see 8.c.vi). The possible bias introduced by this restriction cannot be assessed. It will be assumed that the performance of the medical device is not impacted by this selection bias.

Clinical study data will be monitored to verify its accuracy. Data Management will send out queries to the site in case of inconsistencies, contradictions, suspicious values or missing data.

8. Statistical analysis conventions

a) Analysis Variables

The following variables will be collected by the investigator during the study visits in the **electronic CRF** (eCRF) and will be analysed by means of descriptive statistics:

- Informed consent data (date and time, by whom provided)
- Demographic data (age, gender, childbearing potential, race)
- Vital signs prior to the procedure (weight, height, heart rate, systolic and diastolic blood pressure determination, ECG), during and 24-hours post-procedure (heart rate, systolic and diastolic blood pressure)
- Physical examination prior to the procedure and 24 hours post-procedure (general appearance, cardiovascular, respiratory, skin, eyes, ears, abdomen, genitourinary, neurological, other)
- Timing of stroke onset and admission in hospital
- National Institutes of Health Stroke Scale (NIHSS) and Glasgow Coma Score prior to the procedure
- Medical history data (smoking, alcohol consumption, cardiac and non-cardiac)
- Baseline and 24 hours post-procedure blood laboratory evaluation (haematology, biochemistry, coagulation).
- Performed neuro imaging exams (date, type and findings)
- Eligibility to in- and exclusion criteria
- Timing during the intervention (tPA start (if applicable), anaesthesia start (if applicable), puncture, thrombus removal, revascularization, sheath removal, angio, fluoro, skin closure)
- Investigational, neurovascular and EVT devices used during the intervention (amount, type)
- Number of passes during the EVT intervention
- Procedural characteristics (timing) and events during EVT procedure
- CSGS performance (grading on 5-point Likert scale)
- Usability of CSGS (grading on 5-point Likert scale)
- Clot handling (timing and number of fragments)

- AE, SAE, SADE (diagnosis, seriousness, severity, timing, relationship to procedure/device/EVT, action taken and outcome)
- Concomitant medication (dose, type, frequency, indication, route, start and stop)
- Device Deficiencies (device, timing, description)
- Protocol violations (date and category)
- Suspected etiology
- Results pregnancy test (if applicable)
- Reason for early termination (if applicable)

The following variables will be collected at the histopathological Core Lab, entered in the eCRF and will be analysed by means of descriptive statistics:

- Clot composition (percentage RBC, WBC, fibrin and platelets and others): 3 slices will be analysed, one proximal, one in the middle of the clot and one distal. An average of the composition will be calculated.

The following variables will be collected at the imaging Core Lab and will be analysed by means of descriptive statistics:

- Vessel perforation and/or dissection due to the usage of CSGS comparing baseline imaging with procedural and post-procedural imaging (Non-contrast CT of the brain, CT Perfusion of the brain, Angiography of the brain, MRI of the brain), haemorrhage at baseline, final TICI, distal embolization and embolism in new territory.

b) Analysis Set(s)

Intention To Treat (ITT) Population

All patients who were enrolled, so all patients (or legally authorized representatives) who signed and dated the patient information consent form even though the CSGS was not used in the subject.

The safety analysis (amount of AE) will be performed on the intention to treat (ITT) population.

Treated Population

All patients in which the guidewire went through the sheath, even though if not all eligibility criteria were met or if a device failure occurred or if the clot could not be retrieved.

The primary safety endpoint will be determined on the Treated Population.

Per-protocol Population

All patients from the Treated Population who comply to all eligibility criteria.

No separate analyses will be performed for the per-protocol population.

Development Performance Population

This population includes patients from whom at least 1 acquisition was captured by the CSGS. Data of this population will be used during the development phase to build the different models for classification of RBC-positive and platelet-positive.

Local-Scale Performance Population

This population includes patients for whom data was collected by the CSGS during at least 1 tag in the clot based on expert labelling excluding patients from the Development Performance Population. Data of this population will be used during the validation phase to validate the model built during the development phase.
The analysis of the primary performance endpoint will be performed on data from the Local-Scale Performance Population. Performance assessment during the validation phase will be done only on tags in the clot.

Clot-Scale Performance Population

All patients from the Local-Scale Population of which the clot could be retrieved for histological assessment.

Performance evaluation of the secondary performance endpoints related to the clot scale will be done on the Clot-Scale Performance Population.

Reasons why patients did not undergo the study procedure with the study devices will be listed. A listing will give an overview if patients were involved in a specific population set.

c) Statistical analysis methods

i. Statistical Procedures

A major objective of this study is to evaluate the prediction model(s) of the Clotild® Smart Guidewire System. These prediction models are based on physical properties of the impedance measurements captured by the sensor, complemented with a comparison to suitable labels for each endpoint (a.k.a. phenomenological modelling). The impedance dataset that will be constructed during the clinical investigation should serve to develop the prediction models (model development) and to evaluate their prediction accuracy (model evaluation). Such an evaluation methodology is inspired from Good Machine Learning Practice for Medical Device and avoids any optimistic bias.

Given the necessity to isolate a validation set free of any model optimization to obtain unbiased generalization scores, the dataset will be split into two pieces:

1. **The development dataset**, where model optimizations is being made to arrive at a single particular model trained on the full development dataset.
2. **The validation dataset** that is solely used to assess the performance of the model.

Development dataset

Data of the first interventions will be included in the development dataset. These data will be used so that the model can be fully determined before further patients are enrolled in the validation dataset.

In order to develop the prediction model(s), data from the development dataset are analysed on an ongoing basis.

Validation dataset

After completion of the development phase, data of the following interventions will be used in the validation dataset. The validation dataset shall be used to assess the generalization performance of the model(s) developed during the development phase. When moving to the validation stage, all steps used from input data, i.e. individual impedance spectrums for each electrode pair, to the score prediction shall be fully determined. When moving to the validation phase the model(s) shall be locked, remaining unchanged during the course of the validation phase.

This same procedure of splitting the dataset into two sets (development dataset and validation dataset) will be performed for the primary performance endpoint assessment as well as for the secondary and tertiary endpoint assessments.

Confidence intervals will be computed with the bootstrap.

ii. Listing and descriptive statistics

All original and derived parameters as well as population characteristics will be described. Data will be described using summary statistics as described in the sections below. Frequency counts (number of subjects [n] and percentages) will be made for each qualitative variable. Descriptive statistics (n, mean, standard deviation [SD], median, minimum and maximum) will be calculated for each quantitative variable (unless otherwise stated). In general, all data will be listed, sorted by site and subject, and when appropriate by visit number within subject.

iii. Rounding and decimal places

P-values ≥0.001 will be reported to 3 decimal places; p-values less than 0.001 will be reported as "<0.001". The mean, standard deviation, and any other statistics other than quantiles, will be reported to one decimal place greater than the original data. Quantiles, such as median, or minimum and maximum will use the same number of decimal places as the original data. Estimated parameters, not on the same scale as raw observations (e.g. regression coefficients) will be reported to 3 significant figures.

iv. Statistical significance level

A significance level of 0.05 will be used.

v. Software

Statistical software packages like SAS, SPSS or R will be used.

vi. Handling of missing data

Missing CSGS data

Data captured by the CSGS might be missing or anomalous. Based on the Device Deficiency rate obtained in the first 31 treated subjects, the estimated amount is 40%. This missingness/anomaly might be due to the following reasons:

1. Technical malfunctions, leading to missing data or anomalous data, among which:
    - device anomaly caused by manufacturing anomaly;

- device anomaly due to electrical connection loss during navigation.

2. Use error, leading to:
    - missing data because measurements have not been tagged;
    - anomalous data because the sensor was covered by the microcatheter while the measurement was tagged, as confirmed by the imaging Core Lab on the angiography images corresponding to the tagged measurement.

The criteria to discard anomalous measurements due to technical malfunction include:

- Any acquisition associated with an error code raised by transmitter software (for instance due to loss of communication between sensor and transmitter) will be excluded.
- Electrophysiological measurement from an electrode pair that shows a constant raw digital signal across acquisition frequencies (constant or piecewise constant with a single step) is considered anomalous and will be excluded.
- Electrophysiological measurement from an electrode pair that shows digital saturation consistently from REFERENCE to PRE-CLOT is considered anomalous; all remaining measurements made with this electrode pair will be excluded.
- Anomalies related to invalid reference measurements are reported as too-high dispersion across electrode pairs in the `REFERENCE' measurement. Persistence of the anomaly up to the `PRE CLOT' measurement requires exclusion of all measurements from the concerned electrode pairs.

Since the underlying assumption is completely missing at random, no imputation will be done for missing clot data. To deal with missing data, an attrition rate is taken into account in the sample size calculation.

Missing clot data (histological data)

Clinical experience reports that roughly 40% of clots cannot be retrieved during the procedure. This will lead to missing data for histological assessment. However, over the first 31 treated subjects, 30 clots were collected and analyzed by the histology core lab, which is better than expected and decreases the impact of this potential missing data. Here as well, missingness completely at random is assumed and no imputation will be done for the missing clot data.

Missing patient data

Primary and secondary endpoint data are all captured during the procedure. Follow-up safety data are captured until 24 hours post-procedure. Missing patient data will be rare and assumed completely at random. No imputation of missing patient data will be performed.

Missing and partial Adverse Event dates

The recorded dates for adverse events are important for an accurate tabulation of both events and patients, and required for the following:

1. Defining the peri-procedural or post-procedural algorithm.
2. Designation of unique adverse event occurrences recorded intra-patient.

Completely missing or partially missing adverse event dates will be imputed as follows, after due diligence to obtain accurate adverse event information has failed:

If the adverse event start date is completely missing the adverse event will be considered as having occurred during the study unless it can be determined that the adverse event end date occurred prior to the start of the study procedure. If the adverse event end date can be established as prior to the date of the study procedure, the adverse event will be considered as having occurred prior to the start of the study.

If the adverse event start date is partially missing and the partial date is not sufficient to determine if the event occurred after the start of the study, then the adverse event will be considered as having occurred during the study.

vii. Timing of the analysis and interim analysis

Interim DSMB analyses are planned to be performed after treatment of the first 5 patients, 20 patients and after all patients have been enrolled. These interim analysis only include a safety analysis (see xii). Primary safety endpoint will be assessed as well as the amount, seriousness and severity of (S)AEs and their relationship to the study device, procedure and EVT and device deficiencies. This evaluation will be used by the DSMB to recommend to proceed with the study.

The DSMB could have advised for temporary enrolment suspension if one of the following events occur:

- subarachnoid hemorrhage and/or
- symptomatic intracranial hemorrhage

and further investigation is to be done to assess the severity and the causality to the device if:

- <5 patients were enrolled in the study: each event was to be evaluated by the DSMB chairman. The DSMB chairman had to give feedback within 48hrs to suspend enrolment to further investigate the event or not.
- >5 and <10 patients were enrolled in the study: the study enrolment was to be temporarily suspended if >2 events occured since start of enrolment.
- >10 and <20 patients were enrolled in the study: the study enrolment was to be temporarily suspended if >4 events occured since start of enrolment.

In order to develop the prediction model(s), data from the development dataset are analysed on an ongoing basis.

A final analysis of all study endpoints is planned after all patients left the study, all data is monitored, queried, and the database is locked.

viii. Clinical investigation plan deviations

A deviation from the clinical investigation plan is defined as an event where the clinical investigator or site personnel did not conduct the study according to the clinical investigation plan, ISO 14155:2020 and any national or local regulatory requirements.

Clinical investigation plan deviations are captured in the eCRF and will be listed in the final analysis and include all of the information recorded. This listing will be sorted by clinical site, patient number and the type of deviation. Clinical investigation plan deviations can impact the results of the analysis, therefore, reasons for clinical investigation plan deviations will be screened individually to check if bias could have resulted due to specific clinical investigation plan deviations as missed assessments.

A separate table will be presented for deviations to the in- and exclusion criteria and a detailed listing of patients not fulfilling in- or exclusion criteria will be provided.

ix. Patient accounting and study disposition

A complete accounting of patient participation in the study will be presented in a table entitled 'Patient accounting and final study disposition'. The purpose of this table is to provide an accounting of patients from their entrance into the study through the final visit and to account for the evaluations of patients in the analyses of performance and safety, including reasons for early study termination. The table will display the number and percentage of patients that:

- Were enrolled
- Underwent the study procedure
- Completed the 24 hours post-procedure evaluation
- Discontinued from the study
  - Withdrew Consent
  - Failure to meet inclusion and exclusion criteria
  - Substantial non-compliance
  - Physician decision
  - Adverse event
  - Lost to follow-up
  - Discontinuation by sponsor
  - Other (list exact reason)

This table will be presented by investigation site. The listing with reasons patients did not undergo the study procedure will contain the individual reason. A separate listing sorted by patient number will include the reason for withdraw for all patients who discontinue prematurely.

x. Demographic data

All captured demographic and baseline variables will be displayed in tables for the Treated Population. These tables summarize the patient population with respect to gender, age in years at the time of entry into the study, race, height (cm), weight (kgs.), BMI and vital signs like blood pressure and pulse rate at the time of screening. Results of the physical examination and medical history will be presented in a separate table. The analysis of the baseline blood laboratory evaluation (haematology, biochemistry, coagulation) will be performed by calculating the amount of clinically relevant deviations from normal ranges. Last time seen well, time of stroke symptoms onset, time emergency call, arrival and admission at referral/treatment hospital will be presented in a table.

xi. Prior and concomitant medication

Prior and concomitant medication, especially anti-thrombotic medication, is captured in the eCRF. It will be coded according MedDRA guidelines and will be analyzed descriptively. Concomitant medications refer to all medications taken during the study, including medications continued from the pre-treatment screening period. Prior medications refer to all medications that were started and stopped prior to the first treatment with study device and will not be reported as concomitant.

Medications with missing start and stop dates, or having a start date prior to the start of study procedure and missing a stop date, will be counted as concomitant. Partial dates will be handled as follows:

If the year of the study procedure is < the year of start of concomitant medication AND if the month and day of start of concomitant medication are missing AND if the medication stop date is not prior to the date of the study procedure, then the medication is considered concomitant;

If the year of the study procedure = the year of start of concomitant medication AND if the month of the study procedure is < the month of start of concomitant medication AND if the day of start of concomitant medication is missing AND if the medication stop date is not prior to date of the study procedure, then the medication is considered concomitant.

A summary of the concomitant medications will be presented in a table and all concomitant medication will be listed. Antithrombotic medication given prior to or during the procedure will be listed separately and a table will be added with number of patients having received this type of medication.

xii. Safety analysis

The primary safety endpoint (defined in 4) will be analysed in the Treated Population and presented in a table by means of descriptive statistics (count, percentages and 95% CI).

All Adverse Events will be classified with the Lowest Level Term (LLT) from the MedDRA dictionary (version 24.0, March 2021). All adverse events with onset during the study period will be displayed in summary tables for the ITT population. Tables will show the number of adverse events, the number and the percentage of patients affected by relation to usage of the device or the study procedure or to the EVT procedure (study procedure excluded) or underlying disease. Adverse events will be divided by seriousness. Severity, actions taken and outcome of the adverse events will be displayed in tables. Divisions will be made according to the timing of the event (during procedure, after procedure). All adverse events with descriptions of the event will be listed.

Technical events are defined as device or accessory malfunctions that are not associated with any clinical sequelae. All study site reported technical events will be summarized. The number and percentage of patients experiencing 1 or more technical events will be presented by the event description using counts and percentages. A detailed listing of a device deficiencies and malfunctions will be presented.

xiii. Performance analysis

Descriptive statistics will be presented for the primary performance endpoint, secondary and tertiary endpoints in a table. Definitions of those endpoints are summarized in section 4.

No formal time windows have been set for this study. So endpoints will be reported at the timepoint as captured in the eCRF.

xiv. Study procedure and device accountability

Procedural characteristics, amount of passes, events during EVT procedure and the time segments will be summarized using descriptive statistics. CSGS performance, usability of CSGS and clot handling (timing and number of fragments) will be summarized in a table.

A summary of study device accountability for the ITT population will be presented in a table and a complete listing of all study devices will be provided. This listing will be sorted by clinical site and patient number. Other neurovascular and EVT devices used during the intervention (amount, type) will be summarized in a table and a complete listing of those devices will be provided.

xv. Core Lab data analysis

Histological clot characteristics and results of the imaging Core Lab will be summarized in tables.

xvi. Imaging exams

A summary of all neuro imaging exams captured by the investigator in the eCRF will be presented in a table.

xvii. Clinical evaluation

Results of the NIHSS and Glasgow coma score at screening will be presented in a table. Vital signs during the procedure and the results of the physical examination post-procedure will also be displayed in a table. Additionally, the laboratory results 24 hours post-procedure and suspected etiology will be summarized in a table.

xviii. Sensitivity Analyses

In order to assess the possible impact of missing data on the primary safety endpoint a sensitivity analysis will be conducted. Given the primary safety variable is a binary variable it is rather straightforward to assess the impact of different assumptions. In a worst case scenario all missing data are considered failures which will lead to the worst case scenario estimator of the proportion of perforations/dissections.

9. References

Schober P, Boer C, Schwarte L. Correlation Coefficients: Appropriate Use and Interpretation. Anesthesia & Analgesia: May 2018 - Volume 126 - Issue 5 - p 1763-1768. doi: 10.1213/ANE.0000000000002864

Hanley, J A, and B J McNeil. 1982. “The Meaning and Use of the Area under a Receiver Operating Characteristic (ROC) Curve.” Radiology 143 (1): 29–36. https://doi.org/10.1148/radiology.143.1.7063747.

Good Machine Learning Practice for Medical Device Development: Guiding Principles (FDA, October 2021)

10. Listing of Tables, Listings and Figures

This section is to give precise details for each table, listing or figure to be produced.

Tables

1. Study dates (first patient in – last patient out)
2. Patient accounting and final study disposition
3. Patient compliance to in- and exclusion criteria
4. Patient demographics, baseline data and vital signs
5. Patient physical characteristics at screening
6. Patient medical history
7. Patient laboratory results at screening

8. Timing of stroke onset and admission in hospital
9. NIHSS and Glasgow Coma Score at screening
10. Suspected etiology of stroke
11. Primary safety endpoint
12. Number of SAEs/AEs/SADEs and type, severity, relationship to study device / study procedure / EVT procedure, treatment, outcome
13. Performance analysis: primary performance endpoint and secondary endpoints
14. Tertiary endpoints
15. Procedural characteristics
16. Histological clot characteristics (Core Lab assessment)
17. Imaging Core Lab results
18. Performance of CSGS
19. Usability of CSGS
20. Study device accountability
21. Other neurovascular and EVT devices used during the procedure
22. Patient vital signs and during the procedure and 24 hours post-procedure
23. Physical examination 24 hours post-procedure
24. Patient laboratory results 24 hours post-procedure
25. Summary of concomitant medications
26. Summary of anti-thrombotic medication
27. Summary neuro imaging exams

Listings

1. Patients not fulfilling in- or exclusion criteria
2. Reasons patients did not undergo the study procedure
3. Reasons patients terminate the study prematurely
4. Study device accountability
5. Other neurovascular and EVT devices used during the procedure
6. All AEs
7. Device Deficiency/Malfunctions
8. CIP deviations
9. Anti-thrombotic medication per patient
10. Concomitant medication per patient
11. Overview of patients and their involvement in the different analysis sets.

Figures

1. Disposition of subjects per site and compliance to eligibility criteria.

11. Appendices

Abbreviations and Definitions

Term	Definition
ADE	Adverse Device Effect
AE	Adverse event
AUC	Area under the curve
CSGS	Clotild® Smart Guidewire System
CI	Confidence Interval
CT	Computed Tomography
CTA	Computed Tomography Angiography
DSA	Digital Subtraction Angiography
DSMB	Data Safety Monitoring Board
DMC	Data Monitoring Committee
EC	Ethics Committee
eCRF	Electronic Case Report Form
ECG	Electrocardiogram
EVT	Endovascular Thrombectomy
MRI	Magnetic Resonance Imaging
MRA	Magnetic Resonance Angiography
NIHSS	National Institutes of Health Stroke Scale
RBC	Red Blood Cell
ROC	Receiver Operator Characteristic
SADE	Serious Adverse Device Effect
SAE	Serious Adverse Event
SD	Standard Deviation
tPA	Tissue Plasminogen Activator
WBC	White Blood Cell