Clinical Study Protocol with Statistical Considerations

Randomized Study of TransAeris® system for Enhanced Recovery After Surgery (ERAS) in cardiac surgery patients in France at risk of prolonged mechanical ventilation

Protocol number 20-1000-108 Version D

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Clinical Study Protocol:

Randomized Study of TransAeris® system for Enhanced Recovery After Surgery (ERAS) in cardiac surgery patients in France at risk of prolonged mechanical ventilation

Short Title: ERAS TransAeris® Study

Protocol number: 20-1000-108

Revision: VERSION D

Date: April 19, 2022

Sponsor: Synapse Biomedical - Europe
7 Rue de la Liberation
95880 Enghien Les Bains, France

Investigator: Dr Charles-Henri DAVID
Chirurgien Cardio-thoracique
Service de CTCV - CHU Nantes
02401(68569)
Email: CharlesHenri.david@chu-nantes.fr

PROTOCOL SIGNATURE PAGE

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I have read the protocol and I agree to conduct the study in accordance with the design and specific provisions of this protocol, in accordance with the ethical principles that have their origin in the Declaration of Helsinki, and that are consistent with Good Clinical Practice (GCP) and the applicable regulatory requirements. The study will be performed in accordance with the standard EN ISO 14155:2020 on Clinical Investigations with Medical Devices on Human Subjects, European Medical Regulation MDR 2017/745, any regional or national regulations, as appropriate, and recommendations guiding physicians in biomedical research involving human subjects adopted by the 18th World Medical Assembly, Helsinki, Finland, 1964 and later revisions.

I guarantee that the information and data contained in this document will be considered and treated as strictly confidential: any information contained in it may not be divulged without the prior written approval of the Synapse Biomedical and will be used exclusively for the evaluation and conduct of the clinical trial.

Site Name: _____

Printed Name of Principal Investigator: _____

Signature of the Principal Investigator	Date

Printed Name of Sponsor Representative: _____

Signature of the Sponsor Representative	Date

Revision History

Revision	Release Date	Description
A	29Nov2021	Initial release
B	10Jan2022	Short Title added Section 15 updated as per MDR 2017/745 requirements
C	3March2022	Edits to respond to EC Review (26-Jan-2022) for resubmission to EC
D	19April2022	Edits to respond to EC Review (7April2022) _ CPP Nord Ouest 2

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1. PROTOCOL SYNOPSIS

Title	Randomized Study of TransAeris® system for Enhanced Recovery After Surgery (ERAS) in cardiac surgery patients in France at risk of prolonged mechanical ventilation
Protocol Number	20-1000-108
Device Tested	TransAeris® System
Purpose	This study will be conducted as a randomized trial of the TransAeris system for the prevention and treatment of ventilator-induced diaphragm dysfunction (VIDD) in patients identified prior to surgery to be at greater risk of prolonged mechanical ventilation (PMV).
Study Objectives	The specific objectives of this study are: Primary objective: • Evaluate the credibility of expected treatment benefits Secondary objectives: • Identify safety concerns • Identify barriers to enrollment • Evaluate the randomized protocol in single site setting, prior to multi-center expansion • Assess the capability of collecting the specified evaluation criteria • Assess the capability to quantify diaphragm electrical activity through diaphragm EMG monitoring
Study Design	This study is an open label, randomized, cross-over study in adult open cardiac surgery patients. All subjects will receive TransLoc electrodes at the time of open sternotomy cardiac procedure. Half of subjects will have TransAeris turned on upon entry to ICU, the other half will receive standard of care with TransAeris turned on if still intubated on mechanical ventilation after 120 hours.

Outcome Measures	*Primary Safety: Incidence of Serious Device Related Adverse Events* *Primary Efficacy: Mechanical ventilation time and proportion of patients weaned at 48- and 120-hours post-op* *Ancillary Outcomes:* *Safety:* Interference with ICU care Device related adverse events 30-day mortality *Efficacy:* Time on mechanical ventilation – measured in hours from entry to ICU and reversal of paralytics EMG analysis of cross-over patients Ventilator Free Days Length of stay, ICU & Hospital
Enrollment	30 subjects will be randomized prior to surgical placement of TransLoc electrodes
Participating Site	1 French site will participate in this study
Inclusion Criteria	1. Subject is undergoing an open cardiac procedure by median sternotomy 2. Subject is at risk of prolonged mechanical ventilation according to one or more of the following criteria: a. Prior open cardiac surgery b. Left Ventricular Ejection Fraction (LVEF) ≤ 30% c. History of TIA or CVA d. Pre-operative or anticipated intraoperative intra-aortic balloon pump e. History of COPD 3. Subject is at least 22 years of age 4. Informed consent has been obtained from the subject 5. Subject is covered by a healthcare insurance
Non-Inclusion Criteria	1. Subject is on invasive mechanical ventilation prior to procedure 2. Subject has known or pre-existing phrenic nerve paralysis 3. Subject is having a left ventricular assist device (LVAD) implanted 4. Subject has progressive, non-reversible neuromuscular disease affecting the diaphragm 5. Subject is pregnant or lactating 6. Subject is actively participating in another clinical study which could affect outcomes in this study 7. Subject deprived of liberty 8. Subject under court protection

Estimated Duration of the Study	• Enrollment period estimated: 4 months • Per subject study duration estimated: 1-30 days on device, up to 60 days with 30-day post-explant follow-up • Study completion estimated: 6 months
Sponsor	Synapse Biomedical - Europe 7 Rue de la Liberation 95880 Enghien Les Bains, France

2. ABBREVIATIONS AND ACRONYMS

CIF- Cumulative Incidence Function

COPD – Chronic Obstructive Pulmonary Disease

CVA – Cerebro-Vascular Accident

EMG – Diaphragm Electromyograph

EUA – Emergency Use Authorization

FTW – Failure to Wean

HDE – Humanitarian Device Exemption

HFNC – High-Flow Nasal Cannula

HLOS – Hospital Length of Stay

IABP – Intra-Aortic Balloon Pump

I_C – Cathodic Current

ICU – Intensive Care Unit

IDE – Investigational Device Exemption

LOS – Length of Stay

LVEF – Left Ventricular Ejection Fraction

MV – Mechanical Ventilation

NC – Nasal Cannula

NeuRx DPS – NeuRx Diaphragm Pacing System

NIV – Non-Invasive Ventilation

PMV – Prolonged Mechanical Ventilation

POD – Post-Operative Day

PPV – Positive Predictive Value

PW – Cathodic Pulse Duration

RR – Risk Reduction

SCI – Spinal Cord Injury

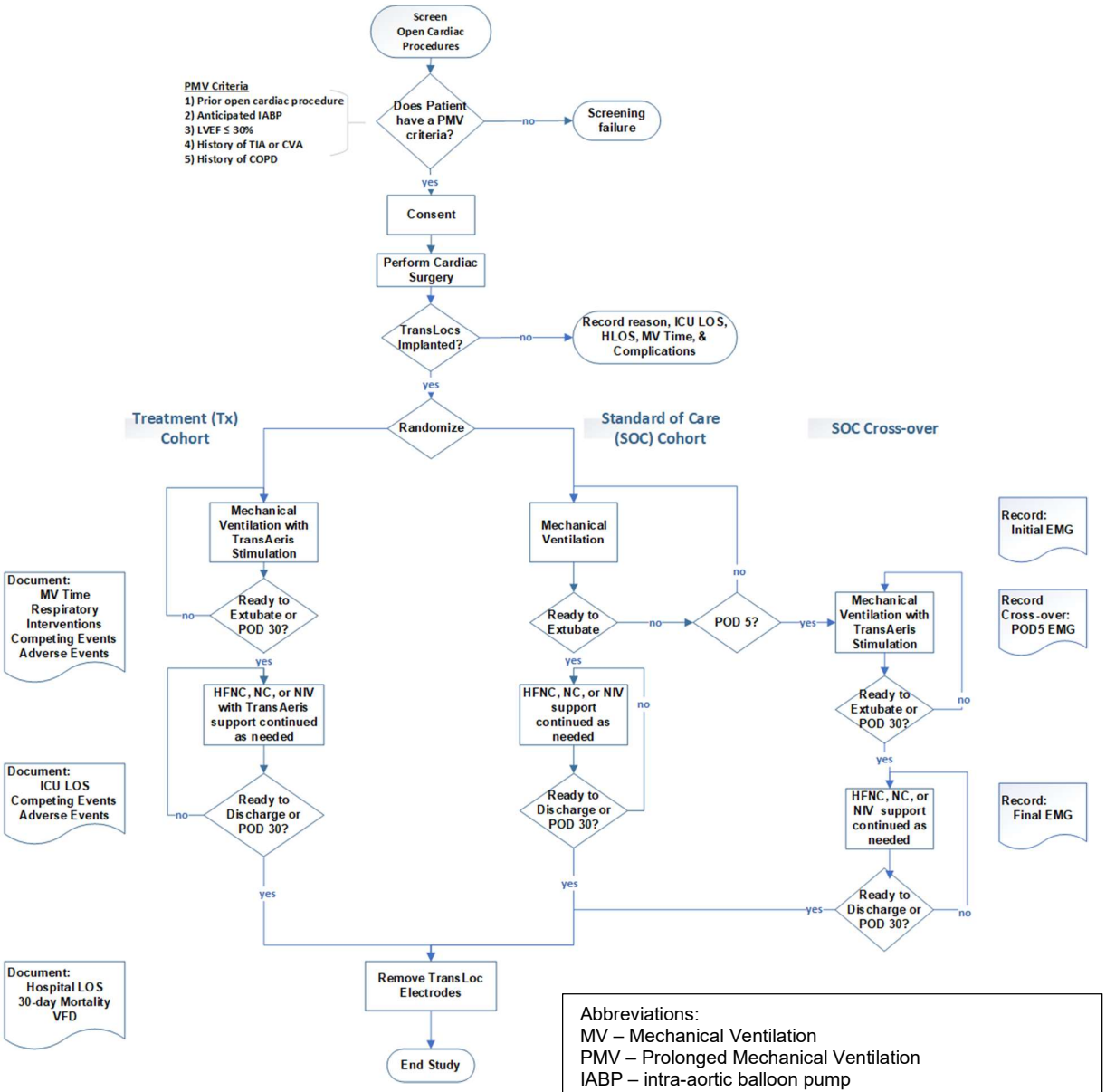
SOC – Standard of Care

STS – Society of Thoracic Surgeons

TIA – Transient Ischemic Attack

VFD – Ventilator Free Day

VIDD – Ventilator-Induced Diaphragm Dysfunction



Abbreviations:
MV – Mechanical Ventilation
PMV – Prolonged Mechanical Ventilation
IABP – intra-aortic balloon pump
LVEF – left ventricular ejection fraction
TIA – transient ischemic attack
CVA – cerebrovascular accident
COPD – chronic obstructive pulmonary disease
ICU – intensive care unit
LOS – length of stay
HLOS – hospital length of stay
HFNC – high-flow nasal cannula
NC – nasal cannula
NIV – non-invasive ventilation
POD – post-operative day
EMG – diaphragm electromyograph
VFD – ventilator free day

Figure 1: Study Flowchart

3. IDENTIFICATION AND DESCRIPTION OF THE INVESTIGATIONAL DEVICE

3.1 Summary Description of Investigational Device and Intended Purpose

The TransAeris® System is a temporary percutaneous intramuscular diaphragm stimulator intended for patients at risk of or on prolonged positive pressure mechanical ventilation. TransAeris is a CE marked medical device and is indicated for use in the prevention and treatment of ventilator-induced diaphragm dysfunction (VIDD).The TransAeris System includes four TransLoc® intramuscular diaphragm electrodes, the TransAeris external stimulator, and the FrictionLoc™ connectors which provide the interface between the TransLoc electrodes and the TransAeris stimulator. Placement of the diaphragm electrodes can be performed in conjunction with another surgical procedure or as the primary procedure. TransAeris is used to provide neuromuscular electrical stimulation to the diaphragm while the patient is on mechanical ventilation to prevent, slow, or reverse diaphragm disuse atrophy and, more generally, to prevent and treat VIDD. Before discharge, the electrodes are removed from the patient.

3.2 Manufacturer of Investigational Device

Synapse Biomedical, Inc.
300 Artino St.
Oberlin, Ohio 44074
U.S.A.
Tel: (440) 774-2488

3.3 Device Name

TransAeris® System Kit REF 20-1002
TransLoc® Electrode Kit REF 20-1004

3.4 Device Traceability

Each device will be labeled as investigational and will include a lot and/or serial number.

3.5 Intended Purpose of Device; Patient Population and Indication

TransAeris is a temporary percutaneous intramuscular diaphragm stimulator intended for patients at risk of or on prolonged positive pressure mechanical ventilation. TransAeris is indicated for use in the prevention and treatment of ventilator-induced diaphragm dysfunction (VIDD).

3.6 Description of Investigational Device

3.6.1 Introduction

See User Manuals REF 77-1000-FR_B & REF 77-1002-FR_C for complete device instructions.

The TransAeris System is a temporary percutaneous intramuscular diaphragm stimulator intended for patients at risk of or on prolonged positive pressure mechanical ventilation (MV). TransAeris is indicated for use in the prevention and treatment of ventilator-induced diaphragm dysfunction (VIDD).

The TransAeris System includes four TransLoc® intramuscular diaphragm electrodes, the TransAeris external stimulator, and the FrictionLoc® connectors which provide the interface between the TransLoc electrodes and the TransAeris stimulator. Placement of the diaphragm electrodes can be performed in conjunction with another surgical procedure or as the primary procedure. TransAeris is used to provide neuromuscular electrical stimulation to the diaphragm while the patient is on mechanical ventilation to prevent, slow, or reverse diaphragm disuse atrophy and, more generally, to treat VIDD. Once the patient is successfully liberated from mechanical ventilation for 48 hours, the electrodes can be removed from the patient.

A cornerstone of the rationale and evidence supporting the use of intramuscular diaphragm stimulation in the treatment of VIDD comes from the clinical experience with Synapse's NeuRx DPS® (IDE (investigational device exemption) G920162, HDE (humanitarian device exemption) H070003). Patients who have been ventilator dependent for months to years due to chronic high-level spinal cord injury (SCI) have undergone a regimen of diaphragm stimulation with the permanently implanted NeuRx DPS to reverse disuse atrophy and re-educate the muscle. Although diaphragm dysfunction was advanced having accrued over months to years, diaphragm function was substantially restored after just a few days of reconditioning with intramuscular diaphragm stimulation.

Because NeuRx DPS is intended for chronic use, the associated PermaLoc® electrode includes a polypropylene barb affixed to the stimulus end of the electrode which promotes fixation of the electrode to the diaphragm. However, TransAeris is intended for temporary inpatient use and thus, to promote its removability, the associated TransLoc® electrode does not include the polypropylene barb. A study (IDE G150040) demonstrated the safety and performance of TransLoc electrodes for (a) intraoperative placement during routine open and laparoscopic procedures, (b) diaphragm stimulation, (c) stability of placement, and (d) removal after temporary in-hospital use.

Based on available data, it is believed that temporary diaphragm stimulation leads, similar to temporary percutaneous epicardial leads placed during cardiac surgery, can be placed in higher risk patients during thoracic and upper abdominal procedures, as the procedure is already occurring near the diaphragm. Alternatively, the electrodes can be placed with a simple standard minimally invasive laparoscopic procedure. These leads, connected to an external pulse generator, provide the ability to stimulate the diaphragm while the patient is on mechanical ventilation to prevent or minimize the diaphragm atrophy, which may reduce the total time on MV thereby reducing the morbidity and mortality associated with prolonged MV use.

3.6.2 Principle of Operation

Diaphragm pacing with the TransAeris platform technology works at neuromuscular junctions and with propagation of action potentials to surrounding motor endplates. The TransLoc intramuscular electrodes are placed at locations on the surface of the diaphragm called the "motor point". The motor points are the locations in the diaphragm in proximity to where the phrenic nerve axons enter and branches into many subsequent axon terminals. Each axon terminal synapses with muscle sarcolemma for individual fiber contraction. By placing two TransLoc electrodes in each hemi-diaphragm at motor points, the greatest number of motor endplates may be stimulated to create a diffuse contraction of the diaphragm. The proximity of the TransLoc electrodes to the motor point allows the spread of the electrochemical reaction and propagating action potentials induced by the stimulus charge to recruit the desired contraction strength without the risk of axonal degeneration or demyelination from contact with the phrenic nerve. The level and coordination of stimulus to each electrode is controlled by the external stimulator to provide a smooth and

sufficient inspiration. Electrical activation of the diaphragm through motor point stimulation conditions the muscle to mitigate disuse atrophy.

3.6.3 TransLoc Electrode

The TransLoc electrode is a unipolar temporary diaphragm stimulation and sensing lead. It consists of a de-insulated multi-filament 316LVM stainless steel electrode (A) (Figure 2), that continues as an insulated multi-filament lead (B) and terminates in a crimped pin (C) at the proximal end. A blue monofilament (D) extends through the length of the electrode and lead and terminates distally in a curved needle (E) and proximally in a percutaneous access needle (F). The curved needle provides insertion of the electrode into the diaphragm muscle. The percutaneous access needle permits exiting the lead through the abdominal or chest wall.

The TransLoc electrodes are for temporary use and are to be removed prior to hospital discharge. The electrodes are stabilized on the skin surface by sutures which should be removed prior to attempting electrode removal. The electrodes are then removed by applying gentle traction to the electrode until it releases from the skin and can be extracted from the body. Each wire should be examined for completeness. Any retained electrode fragments should be documented.

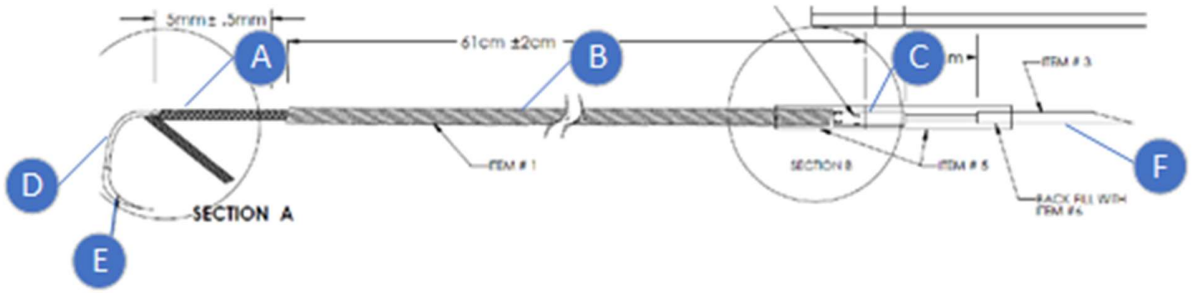


Figure 2: TransLoc Electrode

(Figure labels A-F are described in the section above)

3.6.4 Method of Use

3.6.4.1 Device Implantation

Placement of the diaphragm electrodes will be in conjunction with the primary cardiac procedure and will be placed during the procedure. The implant approach to the diaphragm is from the superior (chest) aspect of the diaphragm surface. From the superior aspect, two electrodes are implanted on the right hemi diaphragm and two electrodes on the left hemi diaphragm. They are implanted next to where the phrenic nerve comes into the central tendon.

Once the desired locations for each of the electrodes are identified, the TransLoc electrodes will be implanted into the diaphragm such that the exposed stimulating surface will be completely within the muscular layers and the blue polypropylene suture extends out of the diaphragm. The blue polypropylene suture will be cut to remove the curved needle from the distal end of the electrode. Adequate electrode length will be left near the diaphragm so that there will not be any tension on the lead during muscle contraction. The process will be repeated for up to four electrodes placed in the diaphragm (two in each hemi-diaphragm).

Each electrode is externalized so that it exits the thoracic cavity in as direct a path as possible to the percutaneous exit site to facilitate easier removal, which is accomplished by using the percutaneous access needle to bring the lead out through the skin in the desired location. The needle is then removed from the lead by cutting through the blue polypropylene suture at the tip of the connector pin.

3.6.4.2 Device Programming

The TransAeris stimulator is programmed for the patient with intensity and frequency set up to elicit diaphragm recruitment without pain or discomfort from the stimulation. The clinician may program the intensity of each channel to optimize diaphragm recruitment. Frequency creates a tetanic or fused diaphragm contraction. Frequency affects all four channels and is set by the clinician to create a fused contraction. The pulse bursting is used to increase patient comfort with the stimulation. The trade-off of burst modulation settings is a reduction in diaphragm recruitment.

The stimulator has four independent channels of stimulation. The stimulation is a charge balanced, capacitively coupled current-regulated waveform. The maximum charge per cathodic phase is 5μC. The charge is calculated as the Cathodic Current (I_C) times the Cathodic Pulse Duration (PW). Stimulus waveforms can be selected as single cathodic pulse followed by the anodic recharge phase or as a burst of four cathodic pulses followed by the anodic recharge. The latter, burst mode stimulation, delivers the cathodic charge at a lower amplitude (1/4 of the single pulse value) and distributes four cathodic pulse at 1ms intervals. The burst mode stimulation may be selected to overcome a patient's reported stimulation discomfort.

3.7 Summary of Necessary Training and Experience to Use Investigational Device

Device programming and use training for all study staff will be provided by Synapse Biomedical. Placement of TransLoc electrodes will be performed by surgeons with prior experience, from the TransLoc feasibility trial (G150040), TransAeris pilot trial (G170294), TransLoc EUA, or will be trained by those surgeons.

4. JUSTIFICATION FOR DESIGN OF CLINICAL INVESTIGATION

4.1 Summary of Relevant Preclinical and Clinical Data

Mechanical ventilation (MV) is a life sustaining treatment in patients who are unable to maintain spontaneous ventilation. In the hospital setting, MV is utilized for treatment of acute respiratory failure, trauma, as well as intra-operatively and during post-operative recovery. Unless required due to comorbidities or operative/postoperative complications, the majority of patients undergoing cardiac surgery are liberated from mechanical ventilation during the first 24 hours after surgery(1). However, a review of the STS (Society of Thoracic Surgeons) Adult Cardiac Database over a 3 year period (2014 - 2017) revealed that of the 883,661 patients with known ventilation time, 109,380 (12.3%) remained intubated and on mechanical ventilation after 24 hours, 72,240 (8.2%) after 48 hours, and 38,120 (4.4%) required MV after 5 days (120 hours) (Figure 3). Failure to wean (FTW) from MV occurs in upwards of 40-45% of patients(2-5) and results in significant morbidity, mortality and health care costs.

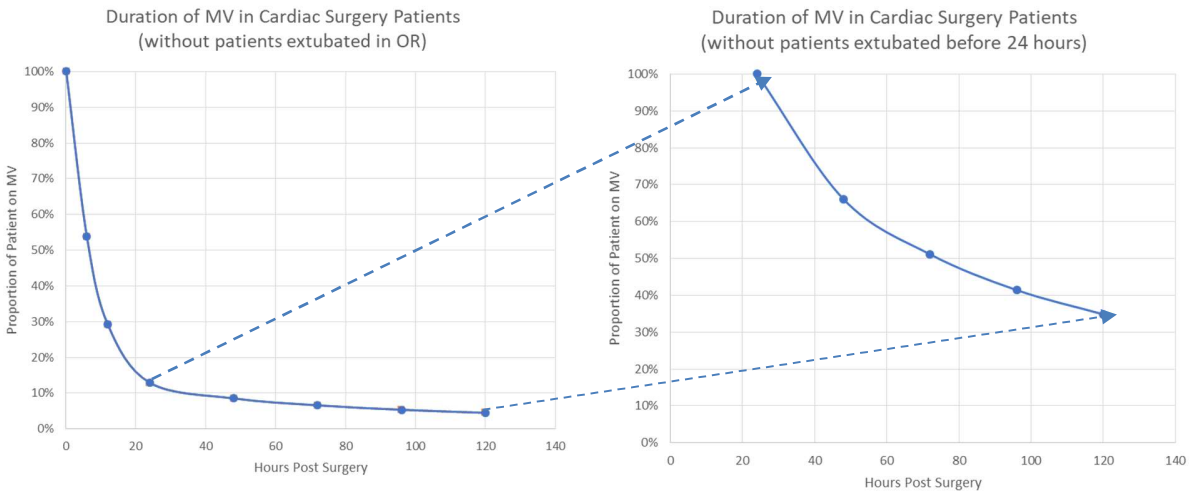


Figure 3. Evaluation group definition graph

The etiology of FTW is multi-factorial, but inspiratory muscle atrophy has been shown to be a significant contributor to this condition(3,5). Research has shown that short exposure to MV leads to decreases in protein synthesis(6) and increased proteoloysis(5,7,8), which is histopathologically manifested as diaphragm muscle atrophy(3). The severity of this muscle atrophy increases with increased time of MV exposure. There is an identified clinical need for treatments to prevent the muscle atrophy that leads to VIDD. Direct electrical stimulation of muscle has been shown to reduce muscle atrophy. The diaphragm pacing system (DPS) has been approved for patients with high spinal cord injuries (SCI) and has been shown to facilitate weaning from MV and be the primary ventilatory support following successful removal from MV(9,10). The current study is designed to evaluate the implementation of TransAeris for the prevention and treatment of VIDD in a surgical population.

The STS performance measures include a quality performance measure (NFQ #0129) for prolonged mechanical ventilation (PMV), defined as the need for intubation greater than 24 hours. The STS and other published data on this study population will provide the basis for comparison of the observed feasibility of safety and efficacy and thus determine the progression to a randomized controlled pivotal study. Current literature(11,12) on PMV illustrates the need for a therapy and identifies that an “enriched study population” based on the risk for PMV may be the most suitable target for an intervention(12).

The initial investigation of TransAeris (IDE G150040) also enrolled in a surgical population. That investigation was primarily focused on placement, stability and removal of the TransLoc electrode during and after the surgical procedure. That study was able to enroll rapidly (twelve patients in under eight weeks) and demonstrated the successful placement, stability and removal of the electrodes in the surgical population.

The next investigation of TransAeris (IDE G170294) focused on enrollment in an “enriched study population” undergoing an open sternotomy cardiac surgery. In this enriched population, meeting identified risk factors for PMV, it was anticipated that the study would provide an apparent visibility to potential benefit. There were 32 patients implanted in this study, 10 received experienced PMV and received TransAeris stimulation. There were no device or procedure related serious adverse events reported in any of the patients. The risk factors for PMV identified five specific criteria that are able to further enrich the study population. Although there is a high incidence of complications and mortality in the targeted high-risk cardiac surgery patient group the patients in this study appeared to benefit from the TransAeris stimulation. Overall, there were

eight of ten patients that received TransAeris stimulation who were successfully extubated within the 30 days or prior to death. At 48 hours post-op, there were 60% of the stimulated patients weaned and at 120 hours there were 70% weaned.

4.2 Application of PICO Model to current study

The PICO model is applied to assess the study construct.
P (Problem / Patient / Population): Ventilator induced diaphragm dysfunction in cardiac surgery patients
I (Intervention): TransAeris stimulation
C (comparison): no stimulation over initial five days
O (outcome of interest): reduction in proportion of cardiac surgery patient requiring MV at 48 hours of stimulation

The Patient population, in general for the intended use, are patients that are at risk of/or experiencing ventilator induced diaphragm dysfunction (VIDD). To study in a more homogeneous population, the patient population is further restricted to cardiac surgery patients. As discussed above, the overall population of cardiac surgery patients has approximately 8% who are on still on mechanical ventilation at 48 hours and 5% who are still mechanically ventilated at 5 days.

To optimize the effect size, we attempted to enrich the study population by introducing the stimulation in patients at higher risk of ≥ 24 hours of mechanical ventilation (MV); the enriched PMV population. These patients represent ≈12% of cardiac patients. The patients requiring 48 hours and ≥5 days of MV from the enriched PMV population is ≈ 66% and 35%, respectively. From the STS 2014 – 2017 Adult Cardiac Database this includes, roughly, 36,460 U.S. patients per year; for this homogeneous enriched population of patients requiring MV for ≥ 24 hours.

The prior pilot study of TransAeris (G170294) identified nine risk factors for PMV as an initial prognostic enrichment inclusion criterion. Based on the resulting MV use of 44 consented patients there were five risk factors identified to be the most significant criteria for determining PMV. They correctly identified 13 out of 14 patients (92.9%) that required PMV as the true positive patients that could potentially benefit from the TransAeris therapy. Conversely, the five risk factors identified 10 out of 29 patients (34.5%) not requiring PMV incorrectly as false positive patients. Defining the positive predictive value (PPV) as the true positive patients (n=13) out of the total positive (n=23) identifications results in a PPV of 56.5%. One additional modification, based on review by cardiac surgeons that participated in the study, the accepted categorization by the American College of Cardiology (ACC), and literature review, is to set the left ventricular ejection fraction (LVEF) accepted for inclusion risk to less than or equal to 30% (LVEF ≤ 30%). This is consistent with the ACC category of “Severe Dysfunction” and is the consistent with the LVEF levels discussed in Hessels, et.al.(12)

The Intervention is the application of the diaphragm stimulation in the enriched PMV population. Of course, to apply the TransAeris intervention, the TransLoc electrodes will have to be placed prophylactically at the time of cardiac surgery. This also reduces the risk of the intervention, as the 2015 feasibility (G150040) safety study showed that the TransLoc electrodes could be safely placed at the time of cardiac surgery, taking less than 5 minutes at the end of the procedure. The pilot study in cardiac surgery patients (G170294) also demonstrated 100% successful electrode removal. The electrodes were able to be removed fully intact in these studies and all other applications of the device, to date.

The Comparator in the model for efficacy in this study is twofold. The primary outcome of this study is to assess the prevention of VIDD through the difference in MV time and proportion of patients weaned at 48-

and 120-hours post-op between the treatment and control groups. The secondary outcome is to assess the treatment of VIDD by crossing over the control patients that remain on mechanical ventilation at 120 hours and observe if there is an effect on the ability to wean those remaining patients with stimulation.

To assess the initial expected outcome, the observational pilot study of TransAeris, resulted in six patients weaned by 48 hours on MV, for a 60% proportion weaned. This compares 34.1% (=1 - 65.9%) weaned from three recent datasets from Suarez-Pierre (2019), Hessels (2019) and the STS Adult Cardiac Database from July 2014 – June 2017.(12,13)

Table 1: Datasets of PMV patients

	Total Patients on MV		
Dataset	at 24 hours	at 48 hours	at 120 hours
Hessels (2019)	229	127 (55.5%)	Not reported
Suarez-Pierre (2019)	1698	1024 (60.3%)	494 (29.1%)
STS (2014 – 2017)	109,380	72,240 (66.0%)	38,120 (34.9%)
TOTAL	111,307	73,391 (65.9%)	38,614 (34.8%)

To power a pivotal study for a 20% risk reduction (RR) in the pivotal study, we would need a total of ≈192 patients randomized. Where the RR is calculated as:

RR = 20% = [Control On MV at 48hrs – Treatment on MV at 48hrs]
= 65.9% - 45.9%

Thus, both groups would have the same rate of patients that would wean prior to 24 hours, based on the prognostic enrichment inclusion criterion, of 43.5% (= 1 – PPV). The TransAeris stimulated group would be anticipated to have 46% of the PMV group still on MV at 48 hours and the control group to have 66% on MV at 48 hours. In the observational pilot we saw 40% on MV at 48 hours. This would translate, in a 40-patient study, to 4 to 5 treatment patients on MV and 6 to 7 control patients on MV at 48 hours. While still anticipated to be statistically inconclusive, with the small sample size requested, as a next step this single-site randomized study is expected to lead into the total 340 implanted patients to yield 192 PMV sample multi-center pivotal study. That is derived from the target 192 patients with PMV and 56.5% positive prediction rate requiring a pool of 340 enrolled patients implanted.

A secondary comparator is to the STS adult cardiac dataset reported number of the enriched PMV group reaching 5 days of MV. As discussed above, we would expect 35% of those patients to still need MV at 5 days. Thus, in a set of 15 control patients, we would expect 2 to 3 patients to still be on MV at 5 days.

Another secondary comparator is the diaphragm EMG activity of the cross-over control patients. It is anticipated that we will see a difference in the bilateral EMG activity from baseline (POD 0) to the point of cross-over at POD 5. It is anticipated that the median frequency, during resting breathing should increase between baseline and the progression of VIDD at POD 5, then reverse back to baseline with TransAeris stimulation. This would be indicative of the use of more glycolytic fibers that have faster conduction velocities with VIDD and then the return of the use of oxidative slow twitch fibers as VIDD is treated with TransAeris.

The intended Outcome of TransAeris for the PICO model is a reduction in time on MV. For this study it is, as stated above for the comparators, a possible reduction in proportion on MV at 48 hours, a potential treatment of VIDD in cross-over patients on MV at 120 hours. Additionally, a difference in EMG analysis between the cross-over patients with more than 120 hours of MV will be assessed as an outcome.

5. RISKS AND BENEFITS OF THE DEVICE AND CLINICAL INVESTIGATION

5.1 Anticipated Clinical Benefits

The benefits of TransAeris stimulation in this subject population are unproven at this time although preliminary evidence suggests that a higher proportion of subjects will be weaned from MV by 48 hours with TransAeris therapy. Subjects may receive no direct benefits from study participation. It is possible that the use of TransAeris may result in the avoidance of prolonged mechanical ventilation due to VIDD, and reduction of MV-associated morbidity. The findings of this study are expected to benefit medical research and advance the development of TransAeris System, which would provide neuromuscular electrical stimulation to the diaphragm while the patient is on mechanical ventilation in the ICU to prevent, slow, or reverse diaphragm disuse atrophy and, more generally, to treat VIDD.

5.2 Anticipated Adverse Device Effects

The theoretical risks related to the study device are listed below, with the observed occurrence in the pilot study:

- Stimulus interferes with heart rhythm; 0 occurrences out of 10 patients
- Stimulus interaction with implanted cardiac defibrillator or cardiac pacemaker; 0 occurrences out of 10 patients
- Pain due to stimulation; 0 occurrences out of 10 patients
- Percutaneous electrode exit site infection requiring antibiotics; 0 occurrences out of 10 patients

The theoretical risks related to the procedure to insert or remove the TransLoc electrodes are listed below, with the observed occurrence in the pilot study:

- Bleeding during surgery due to insertion of the electrode; 0 occurrences out of 32 patients
- Tissue or organ damage during surgery due to insertion of the electrodes; 0 occurrences out of 32 patients
- Breakage of electrode lead; 0 occurrences out of 128 electrodes
- Accidental dislodgement of electrode from diaphragm; 1 occurrence out of 128 electrodes
- Unintentional device fragment retained after removal of electrode; 0 occurrences out of 108 removals
- Bleeding after removal of the electrodes; 0 occurrences out of 32 patients
- Exit site pain after removal of the electrodes; 0 occurrences out of 32 patients

5.3 Possible Interactions with Concomitant Medical Treatments

Warnings regarding possible interactions with other medical devices are included in the warnings section of the User Manuals (REF 77-1000-FR_B & REF 77-1002-FR_C) which is part of this investigational plan. In case of any identified or suspected interaction with other devices used in the care of the patient, the TransAeris stimulation will be ceased. If the interaction is verified, it will be noted as an adverse event, the TransLoc electrodes will be withdrawn and the patients' participation in the study will conclude.

5.4 Risk Mitigation Strategies

Stimulus interference with heart rhythm are mitigated in this protocol per study procedures sections 7.5.1.3.1 and 7.5.1.3.2 which describes cardiac rhythm monitoring. Pain due to stimulation is mitigated through device programming including programmable intensity and frequency settings and the optional burst mode as described in study procedures section 3.6.4.2. Percutaneous electrode exit site infection is mitigated by limiting TransAeris use to the hospital setting where proper exit site maintenance can be ensured. Infection is also mitigated by limiting the TransAeris stimulator to single patient use to prevent transmission of any bacterium from patient to patient on the device.

Additionally, procedure-related risks were mitigated through a prior feasibility study (IDE's G150040 & G170294) which investigated the safety and performance of the TransLoc electrodes including their insertion and removal and investigated the safety and performance of the TransAeris stimulation on open cardiac surgery patients. In total, 176 electrodes were successfully placed in 44 patients during these two pilot studies. Electrodes were placed concomitant to a primary surgical procedure done using one of three approaches: laparoscopic, open abdominal, and open chest. Electrode stability was demonstrated by the collection of daily diaphragm EMG readings. No unretrieved device fragments were left with the removal of electrodes. No device or procedure related adverse events from implantation through electrode removal were reported.

5.5 Risk to Benefit Rationale

Synapse applies the EN ISO 14971 standard for Risk Management in Medical Devices to evaluate identified risks, determine a risk rating and apply our risk acceptance policy. The procedures of analysis, as identified in our standard operating procedures for risk management, are kept up to date with findings from device performance in commercial use, as well as investigational use. Risk mitigation is performed through design practices, protective measures, or informational (instructions for use) with evaluation of these activities on at least an annual basis. An assessment of the detailed evaluation is performed by the Synapse management team and a determination is made if overall residual risk, after mitigation activities, is at an acceptable level. For this clinical investigation, the residual risks have been reviewed by the Synapse management team and the site investigators and determined that they are at an acceptable level. The potential benefits, while they may not apply directly to the individual patients in this study, outweigh the potential risks and the device is safe for human use in the context of the clinical investigation for the proposed intended use.

6. OBJECTIVES AND HYPOTHESES OF THE CLINICAL INVESTIGATION

6.1 Purpose

This study will be conducted as a randomized trial of the TransAeris system for the prevention and treatment of ventilator-induced diaphragm dysfunction (VIDD) in patients identified prior to surgery to be at greater risk of prolonged mechanical ventilation (MV).

6.2 Objectives

The specific objectives of this study are:

Primary objective:

- Evaluate the credibility of expected treatment benefits

Secondary objectives:

- Identify safety concerns
- Identify barriers to enrollment
- Evaluate the randomized protocol in single site setting, prior to multi-center expansion
- Assess the capability of collecting the specified evaluation criteria
- Assess the capability to quantify diaphragm electrical activity through diaphragm EMG monitoring

7. DESIGN OF THE CLINICAL INVESTIGATION

7.1 Type of Clinical Investigation

This study is a monocentric and open label randomized study in adult open cardiac surgery patients. Eligible patients from whom informed consent is obtained are enrolled in the study. All subjects will be implanted with the TransLoc electrodes during their primary surgery. Patients are randomized to treatment or control groups prior to surgical placement of the TransLoc electrodes.

7.2 Outcome Measures

7.2.1 Primary Safety Outcome

Incidence of serious device-related adverse effects

7.2.2 Primary Efficacy Outcome

Time on Mechanical Ventilation and Proportion of patients weaned at 48- and 120-hours post-op

7.2.3 Ancillary Outcomes

Safety:

Interference with ICU care
Device related adverse events
30-day mortality

Efficacy:

Time on mechanical ventilation,
(measured in hours from entry to ICU and reversal of paralytics)
EMG analysis of cross-over patients
Ventilator Free Days
Length of stay, ICU & Hospital

7.3 Investigational Devices and Comparators

Eligible patients from whom informed consent is obtained are enrolled in the study. All subjects will be implanted with the TransLoc electrodes during their primary surgery. Comparison will be made between treatment and control patients during the initial 120 hours of study, then comparison of cross-over control patients' EMG from baseline to treatment. Further comparison of MV Time, ventilator free days and other

outcome measures will be made for all patients, over the course of the 30-day treatment period, to literature on an observational basis.

7.4 Subjects

7.4.1 Inclusion and Non-Inclusion Criteria

The study investigators will screen patients and evaluate their eligibility for this study. Patients will not be enrolled in the study unless they meet all of the inclusion criteria and none of the non-inclusion criteria.

7.4.1.1 Inclusion Criteria

1. Subject is undergoing an open cardiac procedure by median sternotomy
2. Subject is at risk of prolonged mechanical ventilation according to one or more of the following criteria:
    - Prior open cardiac surgery
    - Left Ventricular Ejection Fraction (LVEF) ≤ 30%
    - History of TIA or CVA
    - Pre-operative or anticipated intraoperative intra-aortic balloon pump
    - History of COPD
3. Subject is at least 22 years of age
4. Informed consent has been obtained from the patient

7.4.1.2 Non-Inclusion Criteria

1. Subject is on invasive mechanical ventilation prior to procedure
2. Subject has known or pre-existing phrenic nerve paralysis
3. Subject is having a left ventricular assist device implanted
4. Subject has progressive, non-reversible neuromuscular disease affecting the diaphragm
5. Subject is pregnant or lactating
6. Subject is actively participating in another clinical study which could affect outcomes in this study

7.4.2 Point of Enrollment

30 subjects will receive TransLoc electrodes. Subjects from whom informed consent is obtained are enrolled in the study. Subjects will be randomized to:

Group 1: Tx (Treatment with TransAeris stimulation upon completion of procedure), or;

Group 2: SOC (Control with cross-over to Treatment if still on MV at 120 hours post-op).

7.4.2.1 Randomization

Subject randomization list will be created prior to the start of the study using a block randomization technique. Subject randomization letters will be provided in sealed envelopes in each subject binder. All subject binders will be maintained in the site's clinical research department. Each envelop will be unsealed prior to surgery, and after the subject has signed the consent form.

7.4.3 Subject Withdrawal or Discontinuation

All subjects enrolled in the clinical study shall be accounted for and documented. If a subject withdraws consent before implantation of the TransLoc electrodes, all study-related activities will end. If the subject is implanted with the TransLoc electrodes and withdraws consent, all study-related activities will end and the electrodes will be removed. If the investigator withdraws the subject from the clinical investigation, all study-related activities will end and the reason(s) for withdrawal shall be reported.

7.4.4 Procedures for Replacement Subjects

Subjects that do not have the TransLoc electrodes inserted during their primary surgery will be withdrawn from the study and a replacement will be assigned. The reason for not placing the electrodes will be documented on the Study Exit form for non-implanted patients. Additionally, a chart review will be performed for the non-implanted patients to record their time on mechanical ventilation time, length of ICU and hospital stay and any complications that they experienced during their post-operative stay. Since these patients are consented, this information will be evaluated to determine any relation to their reason for not being implanted to their outcomes and the inclusion criteria.

7.4.5 Enrollment Period and Duration of the Clinical Investigation

It is estimated that enrollment will last approximately four months. The estimated duration of the study from first enrollment to last follow-up is six months. Total study duration per subject is estimated less than 60 days from screening to last follow-up day.

7.5 Procedures

7.5.1 Description of Investigational Procedures

Table 2 lists the data to be collected at each study interval. Study activities are recorded on the case report forms by the clinical coordinator or other clinician designated by the principal investigator. The clinician completing the form signs in the entry designated for completion and the principal investigator reviews all forms and signs in the designated location to indicate review and confirmation of the data entered.

Table 2a: Schedule of Study Activities and Data Collection – Initial All Patients

Activity/Procedure/ Assessment	Screening and Enrollment	At Time of Cardiac Surgery	ICU
Informed Consent	X		
Inclusion/non-inclusion criteria	X		
Demographics, medical history	X		
TransLoc electrode insertion		X	
Concomitant Interventions		X	
Serious adverse events		X	
Adverse device effects		X	

Activity/Procedure/ Assessment	Screening and Enrollment	At Time of Cardiac Surgery	ICU
Device deficiencies		X	
Randomization		X	
Concomitant Interventions			X
Start / Stop date and time of MV use			X
EMG Baseline recording			X

Table 2b: Schedule of Study Activities and Data Collection – Treatment Patients

Activity/Procedure/ Assessment	ICU
Start / Stop date of TransAeris stimulation	X
Competing events	X
Serious adverse events	X
Adverse device effects	X
Device deficiencies	X

Table 2c: Schedule of Study Activities and Data Collection – Control Patients

Activity/Procedure/ Assessment	ICU First 120hrs	ICU At 120hrs	ICU After 120hrs
EMG VIDD Progression recording		X	
TransAeris Start & Settings		X	
TransAeris Start/Stop & Settings			X
Competing events	X	X	X
Serious adverse events	X	X	X
Adverse device effects	X	X	X
Device deficiencies	X	X	X

Table 2d: Schedule of Study Activities and Data Collection – Conclusion All Patients

Activity/Procedure/ Assessment	Discharge	30 Days Post Electrode Removal
Serious adverse events	X	X
Adverse device effects	X	
Device deficiencies	X	
EMG Wean/Discharge recording	X	
TransLoc electrode removal	X	
Follow-up questionnaire		X

7.5.1.1 Screening and Enrollment

Subjects who have been identified prior to surgery to be at risk of prolonged mechanical ventilation after surgery are eligible for this study. Potential subjects will be screened by the investigator for eligibility in the study with respect to the inclusion and non-inclusion criteria (sections 7.4.1.1 – 7.4.1.3). Potential subjects who meet the inclusion and non-inclusion criteria and give informed consent will have demographic, screening, and medical history information including:

- Height
- Weight
- Gender
- Month and Year of birth
- Pregnancy test for women of child-bearing potential
- Admitting diagnosis
- Surgical procedure
- Medical history

Enrollment, demographic information and medical history on documented on case report forms page 1, 2 and 3, respectively.

7.5.1.2 Prolonged Mechanical Ventilation Risk Identification

Prolonged Mechanical Ventilation (PMV) for the purpose of this study is defined as ≥ 24 hours on mechanical ventilation post-surgery. If the patient meets the other inclusion / non-inclusion criteria and has any of the following risk factors for PMV then they will be included if they give informed consent. At least one of the risk factors below must be answered YES on the case report form.

1. Pre-operative or anticipated intraoperative IABP
2. Any prior open cardiac surgery
3. LVEF ≤ 30%
4. History of TIA or CVA
5. History of COPD

The specific risk factors met are to be documented on the enrollment case report form, page 1.

For those undergoing procedures where the STS risk calculator (riskcalc.sts.org) can provide a score, those scores will be calculated and the risk score of PMV for the specific patient will be entered on the case report form, page 2. The risk score and variables will be documented in the case history.

7.5.1.3 Electrode Placement and Device Use

All subjects will be randomized to treatment or control groups prior to implantation of the TransLoc electrodes. Subjects not implanted will be withdrawn from the study. The procedure information is recorded on the case report form, page 4. Specific information concerning the cardiac procedure that the patient is undergoing is recorded on the form as well as procedure time, pump time and any concomitant interventions that the patient undergoes at the time of the cardiac surgery. The device tracking information for the electrodes are recorded in the procedure information as well as an indication of successful electrode placement, or occurrence of an adverse event, competing event or device deficiency.

Patients are randomized to the Treatment or Standard of Care groups as per section 7.4.2.1. The group assignment is recorded on the Post-Op case report form, page 5. This case report form is completed for each patient for each day in protocol, beginning with the day of surgery as day 0. The reversal or time when paralytics are worn off is recorded as well as the current ventilatory support in terms of intubation, extubation, and any type of post-extubation ventilatory support that may be required.

Once the paralytics have been reversed from the surgery, an initial diaphragm EMG is recorded on case report form, page 6. If bilateral EMG is not observed, then a competing event is recorded as described below in section 7.5.1.4.

7.5.1.3.1 Treatment (Tx) Group

TransAeris use begins, in the Tx group as soon as practical. TransAeris stimulation will not begin unless the patient is connected to cardiac monitoring. The cardiac rhythm will be observed for any potential cardiac capture when stimulation is initiated, or any changes are made. The observation of cardiac rhythm interaction is recorded with the stimulator settings on the daily post-op case report form, page 5. TransAeris stimulator settings (stimulus intensity, stimulus frequency, and burst on/off) will be programmed to optimize diaphragm recruitment without compromising patient comfort. Refer to section 3.6.4.2 Device Programming. If stimulation settings cannot be adjusted or the stimulation cannot be applied without eliciting patient discomfort or pain due to the stimulation, then an adverse event indicated on the daily post-op case report form and an adverse event case report form, page 11, should be completed. Stimulation pain should be assessed daily using verbal and/or non-verbal cues from the patient using the visual analog scale shown below. In the event of pain that cannot be eliminated by reducing stimulation intensity then it should recorded as such on the adverse event case report form.

TransAeris stimulation will continue as needed until the patient is successfully weaned. Stimulation may continue beyond successful weaning at the clinician's discretion to provide any additional support to avoid reintubation, but no longer than 30-days post-op.

7.5.1.3.2 Standard of Care (SOC) Group

The SOC group does not receive TransAeris stimulation for the first 120 hours of mechanical ventilation. SOC group patients will not be stimulated unless they are still on mechanical ventilation at 120 hours, at which point they will have an EMG recording made on the case report form, page 6. If intact bilateral EMG is not observed a competing event will be recorded on the competing event case report form, page 10. Otherwise the patient will be identified as a Cross-over patient on the daily post-op case report form, page 5, and follow the process for the Tx group as identified in section 7.5.1.3.1, with the start of TransAeris stimulation.

Any device deficiencies or competing events, that would inhibit or preclude weaning, should be indicated on the daily post-op case report form and the associated detail case report form for device deficiencies, page 12, or competing events, page 10, should be completed for all patients, Tx, SOC or cross-over.

A final EMG recording will be performed prior to electrode removal in all patients. Recordings will be made for a period of at least one minute during quiet, resting breathing or quiet, mechanical ventilation support.

7.5.1.4 Competing Events

At any point during the study both groups will be monitored for competing events that preclude or impede the progression of weaning, i.e. causes other than VIDD that are the source of an inability to wean. These are typically adverse events, unrelated to the device or procedure that will also be recorded in detail, as per Section 15. Identification as a competing event, with time and date of the event, will establish the point of censoring for purposes of analysis of efficacy outcomes. An indication of the occurrence is recorded on the daily post-op case report form (page 5) with more detail of the event, including specific cause of the event and status of the event (as described below) recorded on the competing event detail case report form (page 10). The patient may continue with the TransAeris therapy, if initiated, at the discretion of the clinician.

The event of interest is successful wean from mechanical ventilation and is classified as a Status=0 event. Death prior to successful weaning from mechanical ventilation is a competing event that precludes further weaning and would be classified as a Status=2, right censored event. Other events that hinder the event of interest and alter the probability of occurrence are classified as a Status=1, right censored event. This is depicted below in Figure 3: Competing Event Model.

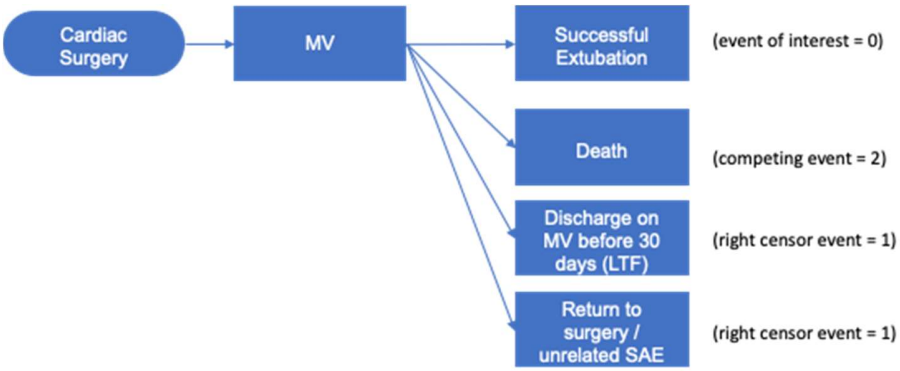


Figure 3: Competing Event Model

The pre-specified competing events and censoring status are provided in Table 3, below. The events that would hinder weaning from mechanical ventilation are right censored with Status 1. Since an intact phrenic lower motor neuron is required for the stimulation to propagate through the diaphragm muscle, bilateral phrenic function, as identified by an observed EMG on both left and right hemidiaphragms, is necessary for the TransAeris stimulation to be effective. Lack of observed bilateral function, indicating a damaged phrenic nerve prior to or during surgery, is considered a competing event that would impede weaning with TransAeris. Additional right censor events may be considered that would meet the definition of precluding or impeding the event of interest (i.e. successful wean) from occurring.

Table 3: Competing Events

Event type	Censor Status	Description	Observed (Suarez-Pierre)
Desired Event	0	Successful wean	39.7%*
Right Censor	2	Death prior to wean, precludes event of interest	20%**
Right Censor	1	Renal failure w/ dialysis initiated post-operatively	9.7%
Right Censor	1	Hemorrhage requiring reoperation	10.4%
Right Censor	1	Delayed Sternal Closure, reoperation	8.3%
Right Censor	1	Sepsis	6.1%
Right Censor	1	TIA or CVA	5.8%
Right Censor	1	Cardiogenic shock, cardiac arrest	13.8%
Right Censor	1	Lack of bilateral diaphragm EMG function	n/a

*Percentage no longer on MV at 48 hours, from Suarez-Pierre (2019) Figure 1
**Approximate 96 hour predicted mortality from Suarez-Pierre (2019) Figure 2

7.5.1.5 Prior to Discharge

The investigator will remove the electrodes prior to ICU discharge or 30 days post-implant procedure, whichever comes first. The electrodes may be withdrawn earlier if the patient has been successfully extubated (and will no longer be at risk of re-intubation). After removal, subjects will be monitored for adverse events prior to ICU discharge or 30 days post-implant procedure, whichever comes first.

7.5.1.6 30 Days Post Electrode Removal

For implanted patients a follow-up questionnaire will be administered by phone, email, or visit to assess at 30 days post-electrode removal the subject's survival, any readmission or reintubation, any post-procedure complications, and subject's location (home, nursing home, long term acute care hospital).

7.5.1.7 Adverse Events and Device Deficiencies

Each subject will be monitored for:

- Serious adverse events
- Adverse device effects
- Device deficiencies (malfunctions, failures, or technical issues)

The investigator and sponsor will assess the incidence of adverse events comparatively between the treatment and control patients. If a significant difference is identified, further study implants will be paused until resolution is identified.

7.5.2 Equipment Used for Study Evaluations

The diaphragm EMG will be recorded with an EMG recorder suitable for recording continuous EMG, such as the Crystal PSG device from Cleveland Medical Devices or other similar device.

7.5.3 Description of Activities Performed by Sponsor Representatives

Synapse Biomedical, Inc. will provide onsite training, technical support, and troubleshooting assistance as needed, including support for TransLoc electrode implantation and device programming and use in the patient room.

7.6 Monitoring Plan

The sponsor, Synapse Biomedical, will monitor the study in accordance with its standard operating procedures to ensure that the study is conducted, recorded, and reported in accordance with the investigational plan, investigator agreement, and applicable EC and regulatory requirements.

Prior to the initiation of the study, the monitor will assess the study site via virtual meeting or physical meeting to assure that the investigator has adequate resources to conduct the clinical study and that the investigator understands his or her obligation regarding: conducting the study in accordance with the agreement with the sponsor, the investigational plan, the EC requirements, and French regulations; obtaining EC review and approval of the clinical study and ensure continuing review; obtaining informed consent; maintaining study records; permitting inspection by French regulators; submitting reports; protocol deviations; investigational device accountability; and financial disclosure, if applicable.

During a monitoring visit, the monitor will review: the continued adequacy of resources to conduct the clinical study; EC approval and other study-related EC records; informed consent records; investigational device storage facilities and accountability practices; investigational device accountability records; correspondence (with the EC, sponsor, or CA, if applicable) including required reports; protocol deviation records; and subject case report forms, adverse device effect reports, and their consistency with source documents.

7.7 Data Protection

All participant data collected during the course of this investigation will be kept strictly confidential. Any information that could identify a subject will remain with the investigator where it will be archived with study documents. Data from all participants will remain pseudo-anonymized for the purposes of data analysis. Data collected for the purposes of this study should be made available for the sponsor/designee at all monitoring visits.

Should the investigation require future review, it may be necessary to allow limited access to the sponsor/designee and regulatory authorities for audit purposes only. They will be granted direct access to the study subjects' original medical records for verification of clinical study procedures and/or data, without violating the confidentiality of the subjects to the extent permitted by the law and regulations.

The sites and the sponsor must follow the General Data Protection Regulation (GDPR) 2016/679, as it applies to the protection and privacy of subject data.

In any presentations of the results of this study or in publications, the subjects' identity will remain confidential.

Study participants and related data will be identified by a unique study identification number. This will not include the participant's contact or identifying information.

The clinical investigation will be registered in a publicly accessible database, on www.clinicaltrials.gov, in accordance with the FDA Modernization Act prior to enrollment of the first subject. Results of the clinical investigation will be made public on this website in accordance with the applicable requirements.

8. STATISTICAL CONSIDERATIONS

8.1 Study Design and Statistical Objective

This study is designed to gather data on the use of TransAeris in subjects identified prior to surgery to be at greater risk of prolonged mechanical ventilation after surgery. Data collected will be compared qualitatively to the relevant clinical literature and used to inform the design and statistical parameters for a subsequent multi-center pivotal trial.

8.2 General Considerations

Continuous data will be summarized using descriptive statistics: mean, standard deviation, median and interquartile range. Categorical variables will be summarized using frequency counts and percentages. For events which can occur more than once in a single subject (e.g., adverse events), the primary analysis will be based on the count of subjects experiencing the event; both patient and event counts will be reported.

All statistical analyses will be performed in STATA version 13.1 or later, or other widely accepted statistical analysis software packages.

8.3 Analysis Population and Significance Testing

The primary analysis will consist of all available data from all enrolled subjects, referred to in ICH E9 ("Statistical Principles for Clinical Trials") as the full analysis set. As the intended analyses of study outcomes

are descriptive in nature, *a priori* hypothesis tests are not proposed; however, any p-values computed and reported will be two-sided, with values less than 0.05 indicating statistical significance.

8.4 Endpoints

8.4.1 Primary Safety Endpoint

The primary safety endpoint is the incidence of serious adverse device effects (SADE), as that term is defined in Section 15.

8.4.2 Primary Efficacy Endpoint

The primary efficacy endpoints are the mechanical ventilation time and proportion of patients successfully weaned at 48- and 120-hours post-op. These will each be evaluated separately for efficacy. A wean will be considered successful if the patient is not reintubated within 48 hours of extubation. The mechanical ventilation time will be the cumulative proportion of time on mechanical ventilation over the 48- or 120-hour period from the entry to the ICU (recorded as ICU Admit Time on the procedure information case report form). If an extubation is attempted but is not successful (i.e., less than 48 hours) then that time will not be considered off of mechanical ventilation. The proportion weaned will be derived from the cumulative incidence function (CIF) of a competing risk analysis.

Data will be analyzed for all patients and using a predictive enrichment threshold of patients on mechanical ventilation. The enrichment strategy is applied to identify patients that may more likely respond to TransAeris stimulation and decrease variability, as per FDA guidance.(14) As the TransAeris treatment is not intended to produce immediate results, but rather is intended to prevent or treat VIDD, patients that are on MV for short periods are expected to have improved spontaneously and thus increase the variability in the outcome measures if included in analysis. Similarly, patients, during the initial hours of mechanical ventilation with paralytic anesthesia still impacting contraction, are not expected to respond to the treatment and thus excluding patients that are weaned from mechanical ventilation during the initial 10 hours post-op will be considered as not part of the predictive enrichment analysis. The data will be considered non-proportional and will be assessed using non-parametric techniques.

8.4.3 Secondary Endpoints

Secondary endpoints are defined as follows:

- All-cause mortality through 30 days
- Mechanical Ventilation time, as the cumulative time on mechanical ventilation from ICU entry and paralytic reversal to successful wean,
- Ventilator-free days, defined(15) as time free from mechanical ventilation during first 28 days, where:
  VFD28 = (28 – Time on MV) if Time on MV is less than 28 days;
  = 0 if Time on MV ≥ 28 days
  = 0 if death within 28 days
- Hospital length of stay (admission to discharge)
- ICU length of stay
- Change in median frequency and total power of the power density spectrum (PSD) analysis of inspirations at baseline, pre-stimulation, and post-stimulation in cross-over control patients

All endpoints will be summarized and reported descriptively to characterize outcomes. No *a priori* hypothesis testing is planned for this study.

8.5 Determination of Sample Size

The purpose of this study is to gather information on safety and effectiveness as a lead-in to a subsequent multi-center pivotal trial, and no therefore formal hypothesis testing is defined *a priori*. For this purpose, implant of up to 30 subjects is deemed sufficient for reasonable precision of estimation of study parameters. Based on prior pilot study (G170294) it is anticipated that there will be consented patients that do not get implanted. These non-implanted patients will be accounted for and documented.

8.6 Interim Analysis

No formal interim analysis for early stopping is specified. Accordingly, any analyses of study results which may be performed during study conduct will not be interpreted to permit early stopping of the study for success, although early stopping for safety is permitted as always.

8.7 Handling of Missing Data

Every effort will be made to reduce the incidence of missing data. The study will be conducted with proper screening of study subjects, complete training of participating investigators, study coordinators and monitors. All subject data that are available on subjects who drop out during the study will be included. Missing data will not otherwise be replaced or otherwise imputed, as the primary analysis is to be conducted on the ICH E9 definition of the full analysis set.

The number and proportion of subjects eligible for and compliant with each follow-up examination will be presented. Subjects who withdraw from the study will be tabulated with reasons for withdrawal.

8.8 Poolability of Outcomes

As no formal statistical hypotheses are to be tested, no formal assessment of poolability will be performed.

9. DATA MANAGEMENT

The sponsor will collect study data using paper or electronic case report forms (CRFs). Paper case report forms are filled out by designated site personnel, reviewed and approved by the investigator, submitted to the sponsor, and entered by the sponsor into a clinical data management system (CDMS). The investigator may be prompted by queries from the sponsor data management personnel or monitors to clarify, correct, or confirm data which are missing, out of range, or illogical.

10. PROTOCOL AMENDMENTS

The sponsor, as per MDR 2017/745 and national regulation, must submit and, where necessary, obtain approval from the EC and CA for all subsequent study amendments, such as protocol changes, IB changes, or changes to the Informed Consent Form.

11. PROTOCOL DEVIATIONS

Deviations to the clinical study protocol are not permitted except as necessary to protect the rights, safety and well-being of human subjects. These deviations shall be reported to the sponsor and as part of the final study report submitted to the EC.

When possible, investigators shall notify the sponsor prior to any deviation from the protocol. All non-emergent deviations will be noted on the appropriate case report form (CRF) and will be reviewed by monitors during monitoring visits.

The sponsor shall review all deviations to determine investigator protocol compliance. Action will be taken to secure compliance if repeated protocol non-compliance is noted. This may take the form of training, other corrective actions or, if necessary, disqualification of the investigator from the study.

12. DEVICE ACCOUNTABLILTY

Investigational devices will only be shipped to qualified investigators. Each study device will be labeled for investigational use only and will contain an identifying lot and/or serial number. The date of receipt, use, disposition, subject identifier, and date of return (as applicable) will be recorded on a device accountability log for each device shipped to the site.

13. REGULATORY COMPLIANCE

This study will be conducted in compliance with current standard ISO 14155:2020 (Clinical Investigation of Medical Devices for Human Subjects – Good Clinical Practice), the ethical principles of the Declaration of Helsinki and European Medical Device Regulation MDR 2017/745, and other applicable regulatory requirements. The study protocol and consent must be approved by the responsible EC and per national regulations. Study activities must not commence prior to receipt of documentation of EC approval and Competent Authority approval by the site and sponsor. The Investigator and study staff must comply with the requirements of the EC.

14. INFORMED CONSENT PROCESS

An informed consent document suitable for use in this study, including the elements of informed consent in conformance with ISO 14155:2020. Iterations of this document must also include the elements of informed consent in conformance with ISO 14155:2020 must be approved by the reviewing Ethics Committee. Subjects must be presented with the most current EC approved version of the consent form for signature and study enrollment.

Country specific consent regulations and language requirements should be followed and incorporated into the consent document.

Prior to enrolling in this trial, the subject will review the informed consent materials with the investigator or appointed designee. The consent form and consent discussion should be in a language the subject understands.

In order to provide consent for study participation, each subject must be fully informed about the investigational device, requirements, risks and benefits, and must sign and date the informed consent form acknowledging that participation is voluntary. The subject will sign and date the approved study-specific

informed consent form prior to any study related procedure or testing being performed. However, historical and standard of care medical tests and records may be used to determine eligibility (e.g. imaging, lab tests).

Subjects may withdraw participation at any time during the investigation without sacrificing their rights as a patient or compromising their quality of medical care.

15. ADVERSE EVENTS, ADVERSE DEVICE EFFECTS AND DEVICE DEFICIENCIES

15.1 Definitions as per ISO 14155:2020 & MDR 2017/745

Adverse Event (AE): Any untoward medical occurrence, unintended disease or injury or any untoward clinical signs, including an abnormal laboratory findings, in subjects, users or other persons, in the context of a clinical investigation, whether or not related to the investigational medical device.

Note 1: This definition includes events related to the investigational medical device or the comparator.
Note 2: This definition includes events related to the procedures involved.
Note 3: For users or other persons, this definition is restricted to events related to the use of investigational medical devices or comparators.

Adverse Device Effect (ADE): Adverse event related to the use of an investigational medical device.

Note 1: This definition includes adverse events resulting from insufficient or inadequate instructions for use, deployment, implantation, installation, or operation, or any malfunction of the investigational medical device.
Note 2: This definition includes any event resulting from use error or from intentional misuse of the investigational medical device.
Note 3: This includes 'comparator' if the comparator is a medical device.

Serious Adverse Event (SAE): Any adverse event that led to any of the following:
a) Death,
b) serious deterioration in the health of the subject, that resulted in any of the following:
   1) a life-threatening illness or injury,
   2) a permanent impairment of a body structure or a body function,
   3) hospitalization or prolongation of patient hospitalisation
   4) medical or surgical intervention to prevent life-threatening illness or injury, or permanent impairment to a body structure or a body function,
   5) chronic disease,
c) foetal distress, foetal death or a congenital physical or mental impairment or birth defect

Note 1: Planned hospitalization for a pre-existing condition, or a procedure required by the CIP, without serious deterioration in health, is not considered a serious adverse event.
Events that do not meet these criteria are considered non-serious.

Serious Adverse Device Effect (SADE): Adverse device effect that has resulted in any of the consequences characteristic of a serious adverse event.

Unanticipated Serious Adverse Device Effect (USADE): Serious adverse effect which by its nature, incidence, severity or outcome has not been identified in the current risk assessment.

Note 1: Anticipated serious adverse device effect (ASADE) is an effect which by its nature, incidence, severity or outcome has been identified in the risk assessment.

Device Deficiency: Any inadequacy in the identity, quality, durability, reliability, safety or performance of an investigational device, including malfunction, use errors or inadequacy in information supplied by the manufacturer;.

Note 1: Device deficiencies include malfunctions, use errors, and inadequacy in the information supplied by the manufacturer including labelling.
Note 2: This definition includes device deficiencies related to the investigational medical device or the comparator.
Use Error: User action or lack of user action while using the medical device that leads to a different result than that intended by the manufacturer or expected by the user.

Note 1: Use error includes the inability of the user to complete a task.
Note 2: Use errors can result from a mismatch between the characteristics of the user, user interface, task or use environment.
Note 3: Users might be aware or unaware that a use error has occurred.
Note 4: An unexpected physiological response of the patient is not by itself considered a use error.
Note 5: A malfunction of a medical device that causes an unexpected result is not considered a use error.

Malfunction: failure of an investigational medical device to perform in accordance with its intended purpose when used in accordance with the instructions for use or CIP, or IB.

Incident: Any malfunction or deterioration in the characteristics or performance of a device made available on the market, including use-error due to ergonomic features, as well as any inadequacy in the information supplied by the manufacturer and any undesirable side-effect.

Serious incident: Any incident that directly or indirectly led, might have led or might lead to any of the following:

- **a)** The death of a patient, user or other person,
- **b)** The temporary or permanent serious deterioration of a patient's, user's or other person's state of health,
- **c)** A serious public health threat

Serious public health threat: an event which could result in imminent risk of death, serious deterioration in a person's state of health, or serious illness, that may require prompt remedial action, and that may cause significant morbidity or mortality in humans, or that is unusual or unexpected for the given place and time..

Note 1: This would include events that are of significant and unexpected nature such that they become alarming as a potential serious health hazard or possibility of multiple deaths occurring at short intervals.

15.2 Causality/Relationship

The investigator and the Sponsor will assess the causality of all adverse events in relation to the research, i.e., the relationship between the AE / SAE and the investigational treatment or any other study-related procedures.

Each SAE will be classified according to four different levels of causality (as per MDCG 2020:10/1):

1) **Not related**: relationship to the device or procedures can be excluded when:

- the event has no temporal relationship with the use of the investigational device or the procedures;
- the serious adverse event does not follow a known response pattern to the medical device (if the response pattern is previously known) and is biologically implausible;
- the discontinuation of medical device application or the reduction of the level of activation/exposure, when clinically feasible – and reintroduction of its use (or increase of the level of activation/exposure), do not impact on the serious adverse event;
- the event involves a body-site or an organ that cannot be affected by the device or procedure;
- the serious event can be attributed to another cause (e.g. an underlying or concurrent illness/ clinical condition, an effect of another device, drug, treatment or other risk factors);
- the event does not depend on a false result given by the investigational device used for diagnosis, when applicable;

In order to establish the non-relatedness, not all the criteria listed above might be met at the same time, depending on the type of device/procedures and the serious adverse event.

2) Possible: the relationship with the use of the investigational device, or the relationship with the procedure is weak but cannot be ruled out completely. Alternative causes are also possible (e.g. an underlying or concurrent illness/ clinical condition or/and an effect of another device, drug or treatment). Cases were relatedness cannot be assessed or no information has been obtained should also be classified as possible.

3) **Probable:** the relationship with the use of the investigational device or the relationship with the procedure seems relevant and/or the event cannot reasonably be explained by another cause, but additional information may be obtained.

4) **Causal relationship:** the serious adverse event is associated with the investigational device or with procedures beyond reasonable doubt when:

- the event is a known side effect of the product category the device belongs to or of similar devices and procedures;
- the event has a temporal relationship with investigational device use/application or procedures;
- the event involves a body-site or organ that a) the investigational device or procedures are applied to or b) the investigational device or procedures have an effect on;
- the serious adverse event follows a known response pattern to the medical device (if the response pattern is previously known);
- the discontinuation of medical device application (or reduction of the level of activation/exposure) and reintroduction of its use (or increase of the level of activation/exposure), impact on the serious adverse event (when clinically feasible);
- other possible causes (e.g. an underlying or concurrent illness/ clinical condition or/and an effect of another device, drug or treatment) have been adequately ruled out;
- harm to the subject is due to error in use;

In order to establish the relatedness, not all the criteria listed above might be met at the same time, depending on the type of device/procedures and the serious adverse event.

15.3 Adverse Event Reporting

The reporting requirements adopted in this study are derived from the international standard on clinical investigations ISO 14155:2020 (“Clinical investigation of medical devices for human subjects — Good Clinical Practice"), Article 80 of the EU MDR 2017/745, and MDCG-2020-10/1:

15.3.1 Investigator reporting Responsibilities

Investigators must report to the sponsor, without delay (immediately) and not later than 3 calendar days after his awareness, all adverse device effects (ADEs), device deficiencies and serious adverse events (SAEs) study subjects experience during participation in this investigation.

Each reported event must be appropriately documented to include the event’s serious criterion, relatedness, treatment, and resolution. All reported events that remain unresolved should be re-assessed until the event resolves or the subject’s study participation is complete. In the event of subject death, the investigator will make reasonable effort to obtain a death summary, autopsy report, etc.

15.3.2 Sponsor’s reporting Responsibilities

1) The sponsor will be responsible for **recording** the following events which have been documented by the investigator:

a) any adverse event critical to the evaluation of the results of the clinical investigation: endpoint events (mortality, ICH, and device/procedure related events) and device deficiencies that adversely affect the subject.

b) Any SAE (Serous Adverse Event)

c) Any Device Deficiency that might have led to a serious adverse vent if appropriate action had not been taken, intervention had not occurred, or circumstances had been less fortunate

d) Any new findings in relation to any event referred to in points (a) to (c)

2) The sponsor will be responsible for **reporting**, without delay to all Member States in which the clinical investigation is being conducted, the following events:

a) any serious adverse event that has **a causal relationship** with the investigational device, the comparator or the investigation procedure or where such causal relationship is reasonably possible

b) any device deficiency that might have led to a serious adverse event if appropriate action had not been taken, intervention had not occurred, or circumstances had been less fortunate

c) any new findings in relation to any event referred to in points (a) and (b).

The sponsor will report to all NCAs where the clinical investigation is authorised to start:

- For all reportable events as described in section 2) above resulting in death or which indicate an imminent risk of death, serious injury, or serious illness and that requires prompt remedial action for other patients/subjects, users or other persons or a new finding to it**:**

=> **Without delay** (**immediately,** but not later than **2 calendar days** after awareness by sponsor of a new reportable event or of new information in relation with an already reported event.

- Any other reportable events as described in section 2) above or a new finding/update to it:
  => **Without delay** (i**mmediately),** but not later than **7 calendar days** following the date of awareness by the sponsor of the new reportable event or of new information in relation with an already reported event.

It is the responsibility of sponsor to inform all investigators in writing within 10 working days if device deficiencies, adverse events, adverse device effects, near-incidents, serious adverse events, serious adverse device effects or unanticipated serious adverse device effects lead to corrective actions (*e.g.* change of IFU).

3) Reporting of serious incidents to the relevant CAs

- The manufacturer will report any serious incident immediately after they have established the causal relationship between that incident and their device or that such causal relationship is reasonably possible and not later than 15 days after they become aware of the incident.
- In the event of a serious public health threat a report shall be provided immediately, and not later than 2 days after the manufacturer becomes aware of that threat.
- In the event of death or an unanticipated serious deterioration in a person's state of health a report shall be provided immediately after the manufacturer has established or as soon as it suspects a causal relationship between the device and the serious incident but not later than 10 days after the date on which the manufacturer becomes aware of the serious incident.

16. SAFETY MONITORING

Synapse will designate a qualified physician to function as an independent Medical Monitor. The Medical Monitor will not otherwise be involved with the study and will act in an advisory capacity to assist the sponsor in monitoring subject safety. The Medical Monitor will review the available information (e.g., case report forms and source documentation) for site reported ADEs. The Medical Monitor will review the investigator classification of whether the event was serious, related to the study device or the procedure, and unanticipated.

17. STUDY EARLY TERMINATION

Synapse will monitor the progression of the trial and may suspend or prematurely terminate either a clinical investigational site or the entire clinical investigation.

Instances which warrant premature study termination or site closure may include:

- Suspicion of unacceptable risk to study subjects
- Serious or repeated deviations on the part of an investigator
- Inadequate subject enrollment or a decision by Synapse to suspend or discontinue development of the device

In the event of suspension or premature termination, Synapse will notify the applicable regulatory authorities in accordance with the regulations and ensure that the EC is notified.

18. REFERENCES

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