Modulation of the motor pathway by Transcranial Pulse Stimulation in people with ALS: a pilot randomized trial

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1.0 TRIAL OUTLINE

Study Title	Modulation of the motor pathway by Transcranial Pulse Stimulation in people with ALS: a pilot randomized trial
Study Registration	NEU-TPS-ALS-01
Study Category	Interventional Investigator sponsored study
Phase	Pilot trial
Background & Rationale	Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease preferentially damaging the motor pathway, but also other cognitive networks, leading to motor paralysis and death in 3-5 years after diagnosis. At present, treatments only delay by few months the progression of the disease (e.g., to ventilatory support). For this reason, ALS in an important unmet need that would benefit from non-invasive neuromodulation therapies aimed to preserve central nervous system (CNS) networks. Transcranial Pulse Stimulation (TPS) is a non-invasive neuromodulation therapy that deliver ultra-short mechanical waves of low intensity to the cortex using neuronavigation. Mechanical waves and ultrasounds modulate neuronal activity by activating mechanoreceptors on the neuronal membrane, changing neuronal excitability (producing either activation or inhibition of excitatory (glutamatergic) or inhibitory (GABAergic) neurons depending on the stimulation parameters. TPS has shown the ability to slow disease progression in patients with other neurodegenerative diseases such as Alzheimer disease and Parkinson Disease, suggesting it may protect the cortical networks from neurodegeneration.
Study Design	Adaptive trial design: Stage 1) Open label study to assess the effects (neuromodulation) of TPS on biomarkers in ALS patients; Stage 2) A randomized, blinded, sham controlled study to assess the efficacy of TPS in patients with ALS
Objective(s)	To assess the efficacy of TPS of the motor cortex on biomarkers and clinical endpoints in patients with ALS
Outcome(s)	<u>Primary</u>: Stage 1. Change in the motor cortex excitability threshold from baseline to week 8. Stage 2. Change from baseline to month 6 in the ALS functional rating scale-revised (ALSFRS-R) total score. <u>Secondary stage 1</u>:

	1. Change in the motor cortex excitability (paired-pulse transcranial magnetic stimulation (ppTMS): motor evoked potential (MEP) amplitudes, resting motor threshold, intra-cortical facilitation (ICF) and short intracortical inhibition (SICI)), from baseline to week 4 2. Change plasma NfL levels from baseline to week 4 and 8 3. Changes in muscle strength, assessed by MRC sum score from baseline to week 4 and 8 4. Changes in hand-held dynamometry from baseline to week 4 and 8 <u>Exploratory stage 1</u> 1. Changes in candidate biomarkers SOD1, Ataxin 2, C90rf72, UNC13A, TDP43, stathmin 2, NfL and NfH levels in extracellular vesicles of neuronal origin (nEVs) collected from plasma from baseline to week 4 and 8 <u>Secondary stage 2</u>: 1. Differences (treated vs sham) and change (from baseline to end of the study (month 6)) in the slow vital capacity (SVC) 2. Differences (treated vs sham) and change (from baseline to end of the study (month 6)) in plasma NfL levels or other biomarkers from exploratory stage 1 if found significant 3. Differences (treated vs sham) and change (from baseline to end of the study (month 6)) in in cortical hyperexcitability (MEP amplitudes, resting motor threshold, ICF and SICI) 4. Changes in muscle strength, assessed by MRC sum score from baseline to end of the study (month 6) 5. Changes in hand-held dynamometry, from baseline to end of the study (month 6) <u>Exploratory stage 2</u> 1. Differences (treated vs sham) and change (from baseline to end of the study (month 6)) in candidate biomarkers levels in nEVs collected from plasma that were informative in stage 1 from baseline to week 4 and 8 <u>Safety endpoints</u>: presence of serious adverse events
Inclusion / Exclusion Criteria	Inclusion: Patients with diagnosis of sporadic ALS (definite or clinically probable) as defined by the World Federation of Neurology revised El Escorial criteria, SVC of 50% or greater of estimated measure and presence of cortical hyper-excitability in the motor cortex as demonstrated by TMS; 21 to 80 years old and male or female Exclusion: patients with fALS (based on medical history) that are unable to tolerate TMS studies or have a contraindication as described below

Measurements & Procedures	1. ALSFRS-R 2. SVC 3. MRC sum score 4. Hand-held Dynamometry 5. Motor cortex excitability by TMS 6. Plasma NfL levels 7. Blood: isolation nEVs and biomarker quantification (SOD1, Ataxin 2, C90rf72, UNC13A, TDP43, stathmin 2, NfL and NfH) 8. Brain MRI for neuronavigation 9. TPS stimulations (6 sessions of 20 min each in 2 weeks)
Study Product / Intervention	Neurolith is a medical device delivering transcranial pulse stimulations under neuronavigation guidance.
Controls	ALS patients allocated to sham stimulations. Sham treated patients will be offered to receive TPS therapy in the Open Label Extension phase (OLE) of the trial for ethical purposes.
Number of Participants & Rationale	Stage 1: n=10 Stage 2: n=40 (randomization 2:1 active vs sham stimulation) Rationale for sample size: this is an exploratory sample likely to be informative for experience in planning for future trials.
Study Duration:	24 months Stage 1: 6 months Stage 2: 3 months recruitment, 6 months randomized phase, 6 months OLE, 3-month analysis and clinical scientific report
Timelines:	Stage 1: ● First Patient First Visit (FPFV): September 1st , 2024 ● Last Patient Last Visit (LPLV): February 1st, 2025 Stage 2: ● First Patient First Visit (FPFV): March 1st , 2025 ● Last Patient Last Visit (LPLV): October 1st, 2026
Study Schedule:	Patients are recruited from the ALS center at Hospital del Mar from patients diagnosed with ALS and referrals provided by the local ALS foundation (Miquel Valls Foundation) Stage 1: V1: informed consent, inclusion/exclusion criteria, TMS cortex excitability, brain MRI (for neuronavigation) can be conducted at any time between Visit 1 and 2; V2: stim; V3: TMS and biomarkers assessment week 4; V4: TMS and biomarkers assessment week 8.

	Stage 2: V1: informed consent, inclusion/exclusion criteria, TMS cortex excitability, brain MRI (for neuronavigation) can be conducted at any time between Visit 1 and 2; V2: randomization and baseline; V3-5: stim, biomarkers and scales; V6-V7: open label extension
Study Center:	ALS center and Neuromodulation center. Hospital del Mar – Pompeu Fabra University, Barcelona, Spain
Statistical Analysis Plan	Considering that this is a pilot study, and no previous data is available for this intervention, a convenience sample size of 10 subjects for the pilot and 40 patients for the randomized phase has been defined. Using the results from the CENTAUR trial[1] in patients with ALS, a sample size of 6 patients per arm provided a 90% to detect differences by week 24 with alpha 0.05. Changes on ALSFRS-R, motor threshold, biomarker levels before and after stimulation will be assessed with ANCOVA test.

A list of abbreviations used in this document can be found in Appendix 12.3.

2.0 TRIAL DESIGN

2.1 Trial Design

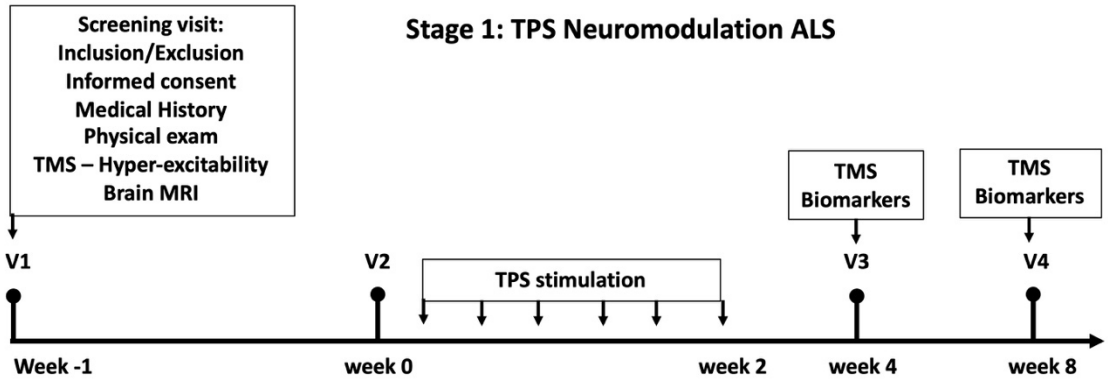
This is a pilot study for evaluating the efficacy of modulating the motor cortex (M1) with Transcranial Pulse Stimulation (TPS)[2, 3] delivered by NEUROLITH device in patients with sporadic Amyotrophic Lateral Sclerosis (ALS)[4]. TPS is aimed to reduce cortical hyperexcitability and slow disease progression of the upper motor pathway, which will reduce the neurodegeneration of the lower motoneuron pathway and delay clinical progression. We will include patients with diagnosis of sporadic ALS (definite or clinically probable) as defined by the World Federation of Neurology revised El Escorial criteria, slow vital capacity (SVC) of 50% or greater of estimated measure and presence of hyper-excitability in the motor cortex as demonstrated by transcranial magnetic stimulation (TMS). Patients 21 to 80 years old and male or female are eligible. Familial ALS is excluded from the study. Clinical adverse events and therapeutic effects will be assessed during the study. Considering the primary endpoint for stage 1 is the efficacy in reducing cortical hyperexcitability, the presence of M1 hyperexcitability at inclusion (baseline) is required.

The study is composed of 2 stages: stage 1 is an open label study to assess the effects of TPS on biomarkers in ALS patients (TMS based hyper-excitability and molecular biomarkers); and stage 2 is a randomized, single blinded, sham controlled study to assess the efficacy of TPS in regulatory accepted clinical scales and biomarkers in patients with ALS.

Specific procedures to be performed during the trial, as well as their prescribed times and associated visit windows, are outlined in the Trial Flow Chart - Section 6.0. Details of each procedure are provided in Section 7.0 – Trial Procedures.

2.2 Trial Diagram

The trial design is depicted in Figure 1.



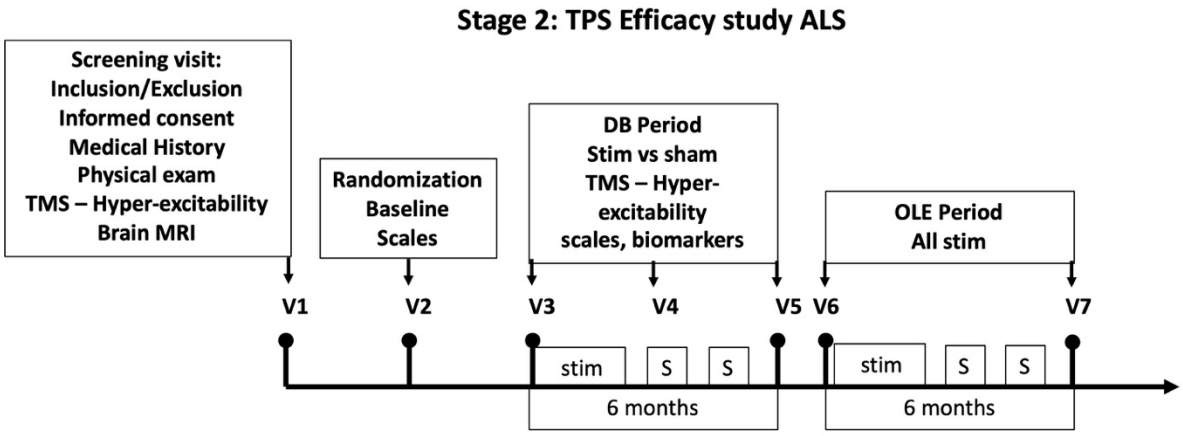


Figure 1 Trial Design Diagram

3.0 OBJECTIVE(S) & HYPOTHESIS(ES)

3.1 Co- Primary Objective(s) & Hypothesis(es)

Objectives:

Stage 1. Change in the motor cortex excitability threshold from baseline to week 8.
Stage 2. Change from baseline to month 6 in the ALS functional rating scale-revised (ALSFRS-R) total score.

Hypothesis:

TPS stimulation delivered to M1 will decrease cortical hyper-excitability and slow disease progression.

3.2 Key Secondary Objective(s) & Hypothesis(es)

Objectives:

Secondary stage 1:
1. Change in the motor cortex excitability from baseline to week 4
2. Change plasma NfL levels from baseline to week 4 and 8
3. Changes in muscle strength, assessed by MRC sum score from baseline to week 4 and 8
4. Changes in hand-held dynamometry from baseline to week 4 and 8

Secondary stage 2:
1. Differences (treated vs sham) and change (from baseline to end of the study (month 6)) in the slow vital capacity (SVC)
2. Differences (treated vs sham) and change (from baseline to end of the study (month 6)) in plasma NfL levels or other biomarkers from exploratory stage 1 if found significant
3. Differences (treated vs sham) and change (from baseline to end of the study (month 6)) in in cortical hyperexcitability (MEP amplitudes, resting motor threshold, ICF and SICI)
4. Changes in muscle strength, assessed by MRC sum score from baseline to end of the study (month 6)

5. Changes in hand-held dynamometry, from baseline to end of the study (month 6)**Hypothesis:**

TPS stimulation delivered to M1 will decrease cortical hyper-excitability and slow disease progression.

3.3. Exploratory endpoints

Exploratory stage 1

1. Changes in candidate biomarkers SOD1, Ataxin 2, C90rf72, UNC13A, TDP43, stathmin 2, NfL and NfH levels in extracellular vesicles of neuronal origin (nEVs) collected from plasma from baseline to week 4 and 8

Exploratory stage 2

1. Differences (treated vs sham) and change (from baseline to end of the study (month 6)) in biomarker levels in nEVs being informative from stage 1 collected from plasma from baseline to week 4 and 8

Hypothesis:

TPS stimulation changes molecular biomarkers levels in nEVs, which represents the cytoplasm content of brain neurons in the previous 12h, as a surrogate endpoint of the neurodegenerative process.

3.4. Safety endpoints

1. Presence of serious adverse events motor cortex excitability, reduce biomarkers of neuronal damage and slow disease progression

Hypothesis:

TPS is safe and well tolerated in patients with neurodegenerative disease and up to 80 years old

4. BACKGROUND & RATIONALE

4.1 Background

4.1.1 Therapeutic Background

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder that involves both upper and lower motoneurons[4]. Patients develop a progressive loss of motor function, with a mean survival of 3 to 5 years. Other brain circuits are being damaged such as frontal-temporal pathways leading to cognitive impairment and even to dementia. There is no diagnostic biomarker, and its diagnosis is based on clinical criteria, which are periodically reviewed and updated[5-7] . ALS is a heterogenous disease from the clinical, genetic, and even histopathological point of view[8] . Furthermore, there are no prognostic biomarkers that predict the outcome of the patients. New tools have even been developed to predict the progression profile of patients (ENCALS survival model, and the ENCALS risk profile) based on gender, age of onset, form, presence or absence of frontotemporal dementia, as well as respiratory function[9, 10] . Data from sporadic and genetic forms of the disease has taught us that several key cellular and molecular pathways are involved in the disease such as cytoskeletal dynamics, neuroinflammation, mitochondrial dysfunction, reticulum endoplasmic stress and protein homeostasis[11, 12] .

Neuromodulation is intended to activate or restore specific neuronal circuits using different types of energy at varying frequency. Invasive neurostimulation by means of deep-brain electric stimulation for Parkinson's disease and Essential Tremor have already achieved the level of validated therapies with high efficacy success and widespread usage. By contrast, the level of validation and benefits provided by non-invasive neurostimulation is very limited. For years, magnetic, electric, or light neurostimulation has been tested but has shown significant limitations because of lack of spatial resolution or low penetration into structures deep in the brain, where relevant motor circuits are located[11].

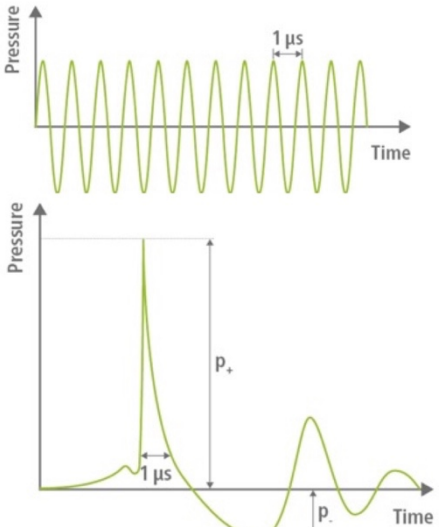


Fig.1. Ultrasound waves vs TPS waves

Transcraneal Pulse Stimulation (TPS) has been shown to provide an adequate neurostimulation platform because it offers high spatial and temporal resolution, as well as the excellent safety based on decades of experience with TPS in osteo-muscular, heart and kidney diseases[2, 3, 13, 14]. TPS can be generated with electrohydraulic, electromagnetic, and piezoelectric devices. Whereas ultrasounds uses an oscillatory sound wave, TPS uses a single sound wave (**Figure 1**). TPS can achieve significant penetration (8 cm) into the brain because of the lower skull absorption, allowing to activate the cortex, including regions further from the skull such as the hippocampus, insula or cingular cortex (**figure 2**)[13]. TPS can activate neurons by microcavitation due to the sound ultrashort waves. Such membrane displacements activate the many mechanosensors found in neurons and other cells.[15] The activation of mechanosensors opens ion channels that change neuronal excitability and therefore induce either activation or

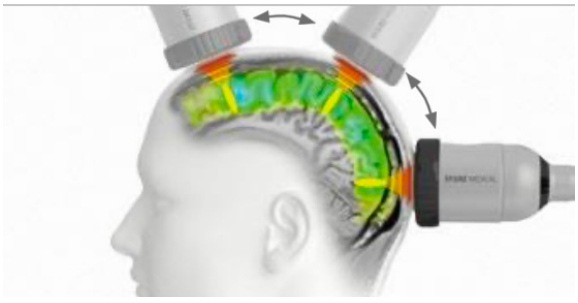


Fig.2. TPS beam penetrates 8 cm in the brain covering the cortex thickness

inhibition of neuronal circuits depending on the TPS protocol and the neuronal cell type[16]. For all these reasons, TPS has become a very promising technology for modulating neuronal circuits safely and effectively for treating brain diseases such as ALS[17-23].

4.1.2. TPS neuromodulation therapy

The TPS neuromodulation device (NEUROLITH) is a medical device for offline MRI-guided neuromodulation treatment that maintains brain region specific targeting based on a neuronavigation system using an electromagnetic TPS system (**figure 3**). The Neurolith system has been approved in Europe (CE mark) in 2018 for the treatment of Alzheimer disease. At present, around 10,000 patients with Alzheimer disease have been exposed to TPS with excellent safety. No serious adverse events have been reported and the most common adverse events are headache or fatigue that disappear in 24-48h. The device directly delivers the ultrashort sound beam to a key structure involved with motor modulation such as M1 cortex using a neuronavigation system (figure 2). The NEUROLITH device will be comfortably applied at the clinic visit where the patient remains sited, the hair will be humidified, and ultrasound gel is applied to the electromagnetic stimulator. The device produces a characteristic sound that is not disturbing but recognized. No heat, pain, numbness or any other sensation is perceived by the patient. The system provides a sham stimulation option based in the use of a membrane containing gas that blocks sound transmission, preserving the characteristic sound or any other feature the patient may notice.

Fig.3. Neurolith device is composed by the stimulation system (control unit and power source) and neuronavigation system

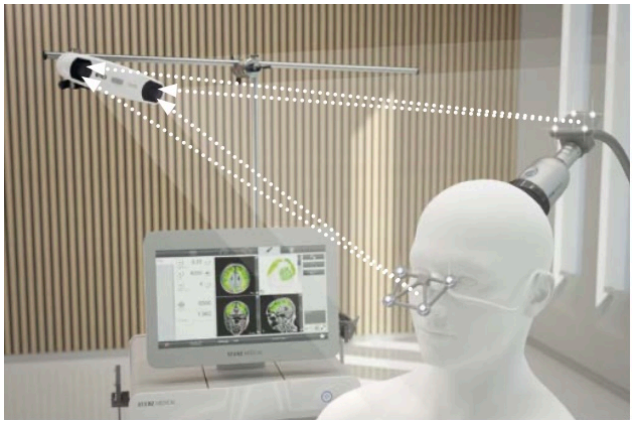


Fig.4. The neuronavigation system guides TPS stimulation using off-line MRI guidance

Prior to first use, every subject will undergo an MRI scan to register the location on their temple region relative to the brain targets for tailored anatomical registration (**figure 4 and 5**). NEUROLITH has an external control unit containing a battery supply for initial cost efficiency, flexibility in stimulation protocols and human clinical study designs.

The stimulation involves delivering 1,000 pulses per cortical region, with a maximum of 6,000 pulses per session[13]. The system uses a low energy flux density maximum of 0.25 mJ/mm2 accordingly with EMA and FDA guidelines (high energy is defined as > 0.5 mJ/mm2). Each session use to extend for 20 min. The standard therapy involves 6 sessions in 2 weeks. After completion, single follow-up sessions are applied every 2 months.

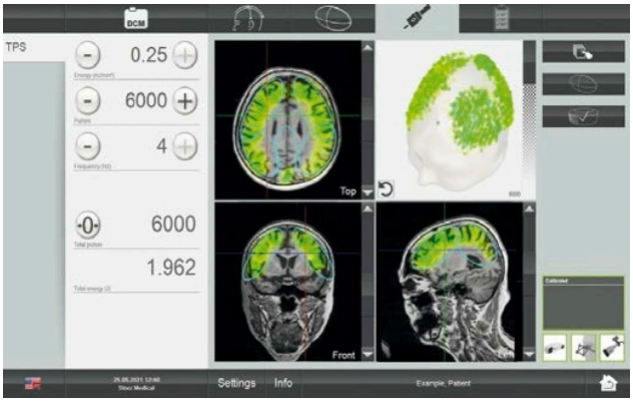


Fig.5. The software provides controls for intensity, pulse number, pulse repetition, total number of pulses and neuronavigation

4.1.3. TPS safety and operation specifications

TPS stimulation of the brain has been shown to be safe and well tolerated in animal and studies and clinical practice. So far, round 10,000 patients with Alzheimer disease have been treated with TPS (6 sessions in 2 weeks) and 800 have been retreated during follow-up (range of re-treatments 3-5) without having observed any serious adverse event[13]. Common adverse events are headaches and fatigue that disappears in 24-48h.

In addition to TPS, the field of ultrasound stimulation is providing significant amount of data supporting the safety of sound waves for neurostimulation. Multiple animal studies have analyzed histological changes of tissue following repeated ultrasound exposure within the FDA-recommended imaging limits and, to date, no studies have found evidence of tissue damage[19, 20]. A more recent study examined long term effects of ultrasound neuromodulation in the brain using nonhuman primates[21]. The researchers treated animal's brains with focused ultrasound across 129- 147 sessions over the course of two years at a semi-regular interval. Across the study, there were no signs of behavioral degradation while performing tasks associated with the treated brain region. Furthermore, comparison of MRI brain scans before and after the two-year treatment showed no morphological abnormalities in these animals.

In addition to large animal models, histological and report-based safety data has been obtained from human neuromodulation studies. Patients scheduled for anterior temporal lobe resection for epilepsy treatment had their temporal lobe exposed to a battery of focused ultrasound paradigms prior to surgical removal[22]. Examination of the excised tissue revealed no indications of morphological damage or neuronal damage (acidophilic cells). In a study with ultrasound parameters comparable to those proposed here in our First-in-Human study, subjects' thalamic nuclei were stimulated to reduce pain perception. The study found a significant reduction in perception and, critically, no adverse events were reported.

4.2 Rationale

4.2.1 Rationale for the Trial and Selected Subject Population

NEUROLITH is a non-invasive neuromodulation device, using low intensity TPS (LITPS) to stimulate neurons involved in motor control. LITPS stimulates neurons by cavitation that activates mechanoreceptors that modulates neuronal excitability and firing. LITPS is safe and can penetrate the skull and brain reaching the whole cortex, including deeper regions such as the hippocampus, insula or cingulate cortex. Therefore, LITPS is a highly selective neurostimulation approach to modulate relevant neuronal networks, in this case the networks regulating the motor circuits. Indeed, LITPS has shown extended effects along time[24].

Given the potential efficacy of LITPS, and the need for highly effective and safe treatments for ALS, this study aims to examine the safety and efficacy of LITPS to modulate motor cortex M1 in patients with ALS.

4.2.2 Rationale for Stimulation Protocols Selection/Regimen

The stimulation protocols for the current study will be 20 min duration as indicated in the CE mark approval. The system uses a low energy flux density maximum of 0.25 mJ/mm2 accordingly with EMA and FDA guidelines (high energy is defined as > 0.5 mJ/mm2). The standard therapy involves 6 sessions in 2 weeks as indicated in the CE mark. After completion, single to multiple follow-up sessions can be applied every 2-3 months.

4.2.2.1 Rationale for the Use of Sham

In order to assess the efficacy of the therapy in clinical outcomes, comparison between active and sham treatment is required. Using sham or placebo for 6 months is accepted for patients with ALS as shown in the most recent phase 2 and 3 trials. In order to encourage participation and fulfill patients' expectations, sham treated patients will receive active stimulation during the open label extension period (OLE), intended to collect more safety data and to assess sustainability of the efficacy observed in the double-blinded period (DBP).

4.2.2.2 Starting Stimulation Protocols for This Trial

The stimulation protocol of TPS in this trial will start at 0.25 mJ/mm2 as defined in the CE mark approval.

4.2.2.3 Maximum Dose/Exposure for This Trial

The stimulation will be always below the threshold that defines high intensity TPS (0.5 mJ/mm2) and below the threshold defined in the CE mark (0.25 mJ/mm2).

4.2.3 Rationale for Endpoints

4.2.3.1 Efficacy Endpoints

<u>Primary endpoints:</u>

Stage 1. Changes in the motor cortex excitability determined by TMS: neuronal hyper-excitability has been described in neurodegenerative diseases including ALS. The hyperexcitability is consider a consequence of the pathogenic process that contributes to neurodegeneration. For this reason, restoring neuronal excitability to normal limits is considered as a therapeutic target

Stage 2. Change from baseline to month 6 in the ALS functional rating scale-revised (ALSFRS-R) total score.: Changes in the ALSFRS-R is accepted primary endpoint by regulatory authorities (EMA and FDA) for defining therapeutic efficacy in this condition.

Secondary endpoints:
Secondary stage 1:
1. Change in the motor cortex excitability from baseline to week 4: to assess changes in excitability in a shorter period of time (4 weeks)
2. Change plasma NfL levels from baseline to week 4 and 8
3. Changes in muscle strength, as assessed by MRC sum score and hand-held dynamometry, from baseline to week 4 and 8

Exploratory stage 1
1. Changes in candidate biomarkers SOD1, Ataxin 2, C90rf72, UNC13A, TDP43, stathmin 2, NfL and NfH levels in extracellular vesicles of neuronal origin (nEVs) collected from plasma from baseline to week 4 and 8: levels of proteins related with ALS pathogenesis in the cerebrospinal fluid (CSF) are proposed as biomarkers of the disease. nEVs is a promising strategy to identify new biomarkers. Such vesicles can be isolated from plasma (even frozen plasma) and reflects the cytoplasm content of the neuronal population 12h in advance[25]. The vesicles content is representative of the cytoplasm and includes proteins, RNAs and metabolites. Then, nEVs can be considered as a surrogate approach to a liquid biopsy of the brain. As such, several biomarkers have been identified in patients with ALS[26], Alzheimer disease[27, 28], Parkinson disease[29, 30], traumatic brain injury or MS[31]. Here, we propose collecting nEVs to conduct a biomarker search study to identify biomarkers associated with the TPS therapy in the motor cortex of people with ALS. Using nEVs, we can assess such biomarkers from a blood draw sample and reflecting their content in the brain, becoming a powerful approach to validate new biomarkers for ALS and response to therapy

Secondary stage 2:
1. Differences (treated vs sham) and change (from baseline to end of the study (month 6)) in the slow vital capacity (SVC)
2. Differences (treated vs sham) and change (from baseline to end of the study (month 6)) in plasma NfL levels or other biomarkers from exploratory stage 1 if found significant
3. Differences (treated vs sham) and change (from baseline to end of the study (month 6)) in in cortical hyperexcitability (MEP amplitudes, resting motor threshold, ICF and SICI)
4. Changes in muscle strength, assessed by MRC sum score from baseline to end of the study (month 6)
5. Changes in hand-held dynamometry, from baseline to end of the study (month 6)

Exploratory stage 2
1. Differences (treated vs sham) and change (from baseline to end of the study (month 6)) in biomarker levels in nEVs collected from plasma that were informative in stage 1 from baseline to week 4 and 8: same as above

4.2.3.2 Safety Endpoints

The safety and tolerability of TPS will be continuously assessed throughout the study via Adverse Event reporting, and vital signs will be assessed at every trial visit. The Columbia-

Suicide Severity Rating Scale (C-SSRS) will be conducted at the baseline and end of the treatment to prospectively assess for the emergence of suicidal ideation and behavior.

4.2.3.3 Exploratory Endpoints

4.3 Benefit/Risk

Participants in this study are patients with ALS, which due to the seriousness of their condition, have agreed to participate. TPS is not invasive, is well tolerated and is a minimal-risk device. As a result, the risk/benefit should be highly beneficial for the participants.

5.0 METHODOLOGY

5.1 Entry Criteria

5.1.1 Diagnosis/Condition for Entry into the Trial

Male and female subjects aged 21 to 80 years (inclusive) with sporadic ALS will be enrolled in this trial. Familial ALS (fALS) accounts for 10% of cases, with sporadic ALS (sALS) accounting for the remaining 90% of cases. Distinction between fALS and sALS will be done by family history.

5.1.2 Subject Inclusion Criteria

In order to be eligible for participation in this trial, the subject must:

1. Be between 21 to 80 years of age (inclusive) on the day of signing informed consent.

2. Patients with diagnosis of sporadic ALS (definite or clinically probable) as defined by the World Federation of Neurology revised El Escorial criteria)[5], SVC of 50% or greater of estimated measure and presence of cortical hyper-excitability in the motor cortex as demonstrated by TMS.

3. Each subject must sign the informed consent form, **in accordance with local requirements**, after the scope and nature of the investigation have been explained to the subject, and before Screening assessments.

4. Based on the investigator's judgment, the subject should:

   Be able to speak, read, and understand the language of the trial staff and the informed consent form;

   Possess the ability to respond verbally to questions, follow instructions, and complete study assessments;

   Be able to adhere to the stimulation protocol and visit schedules.

5. Have results of a physical examination (PE), including neurological exam and vital signs not clinically significant.

6. If female, following actively anti-conceptive measures or not be of childbearing potential as indicated by one of the following:

- Has reached natural menopause, defined as 45 years of age with ≥6 months of spontaneous amenorrhea
- Has had a hysterectomy;
- Has had bilateral tubal ligation; or
- Has had a bilateral oophorectomy (with or without a hysterectomy) and greater than 6 weeks have passed since the surgery

9. Male participants must ensure a condom is used for all sexual intercourse as well as following the acceptable methods of contraception listed above for your female partner. You must ensure that they are used for the entire duration of the study. You should not father a child or donate sperm for this same period.

5.1.3 Subject Exclusion Criteria

The subject must be excluded from participating in the trial if the subject:

1. Patietns with familial ALS
2. Has any unstable medical or psychiatric disease other than ET
3. Patients that are unable to tolerate TMS studies.

Additional exclusion criteria include:

1. Has evidence of a clinically relevant neurological disorder at Screening, including but not limited to: cerebellar degeneration, hyperthyroidism, multiple sclerosis, vascular dementia, Alzheimer's disease (AD), frontotemporal dementia, Huntington's disease, progressive supranuclear palsy, neurosyphilis, dementia with Lewy bodies, other types of dementia, mental retardation, hypoxic cerebral damage, cognitive impairment due to other disorders, or history of head trauma with loss of consciousness that either led to persistent cognitive deficits or in the opinion of the investigator confounds the diagnosis.
2. Is at imminent risk of self-harm, based on clinical interview and responses on the Columbia-Suicide Severity Rating Scale (C-SSRS), or of harm to others in the opinion of the investigator. Subjects must be excluded if they report suicidal ideation with intent, with or without a plan or method (e.g., positive responses to items 4 or 5 in assessment of suicidal ideation on the C-SSRS) in the past 2 months or suicidal behavior in the past 6 months.
3. Has a history of alcoholism or drug dependency/abuse within the last 5 years of Screening.
4. Has a recent history (within the 6 months prior to Screening) of regular consumption (3 or more days per week) of more than 3 alcoholic beverages per day
5. Has a recent or ongoing, uncontrolled, clinically significant medical condition where participation in the trial would pose a significant medical risk to the subject or would confound study results within 3 months of Screening, such as:
    - Conditions including but not limited to diabetes, hypertension, Human Immunodeficiency Virus (HIV) or other relevant infections, thyroid or endocrine disease, Chronic Obstructive Pulmonary Disease (COPD), delirium, congestive heart failure, angina, cardiac or gastrointestinal disease, or renal disease requiring dialysis.
    - Major surgery including not limited to abdominal, thoracic, cardiac, or orthopedic surgery, or any procedure requiring general anesthesia
    - Subjects with recently controlled or treated medical conditions may be considered for re-screening on a case-by-case basis.
    - Has a history of hepatitis or liver disease that, in the opinion of the investigator, has been active within the 6 months prior to Visit 1.
6. Has a history of malignancy occurring within the 5 years immediately before Screening, except for a subject who has been adequately treated for:
    - Basal cell or squamous cell skin cancer;
    - In situ cervical cancer;
    - Localized prostate carcinoma;

- Who has undergone potentially curative therapy with no evidence of recurrence for ≥3 years post-therapy, and who is deemed at low risk for recurrence by her/his treating physician.

7. Is pregnant, is attempting to become pregnant, or is nursing children.
8. Is or has an immediate family member (e.g., spouse, parent/legal guardian, sibling, or child) who is investigational site directly involved with this trial.

5.2 Trial Treatment(s)

The investigator shall take responsibility for and shall take all steps to maintain appropriate records and ensure appropriate supply, storage, handling, distribution, and usage of trial treatments in accordance with the protocol and any applicable laws and regulations.

5.2.1 Therapeutic regimen Selection: TPS stimulation protocols

20 min stimulation with a maximum of 6,000 pulses and maximum of 0.25 mJ/mm2 per session. 6 Sessions in 2 weeks per treatment period followed with a single stimulation session every 2 months (2 additional sessions by month 2 and 4).

5.2.2 Timing of Therapeutic Regimen Administration

The treatment will be administered in the first 4 weeks after randomization with a duration of 2 weeks for the first period. Then, single stimulation sessions will be conducted every 2 months, which means 2 additional sessions (month 2 and 4).

5.2.3 Trial Blinding/Masking

Patient and physician will be blinded. The sham reproduces the patient experience of the TPS treatment (mainly the sound produce by the stimulator). The treating researcher will be unaware of the patient allocation. An independent investigator informed of the therapeutic arm will prepare the device to administer treating stimulation or sham and will record such assignment for each treating visit in a secured file not available for the treating researcher.

5.3 Randomization

Treatment allocation/randomization will occur centrally using an interactive voice response system / integrated web response system (IVRS/IWRS). There are 2 treatment arms: active TPS and sham. Subjects will be assigned randomly in a 1:1 ratio to either TPS or sham.

5.4 Stratification

No randomization stratification is considered in this study

5.5 Concomitant Treatments (Allowed)

Patients will be allowed to take their therapy prescribed for ALS and any other comorbidity.

Treatments specifically prohibited in the exclusion criteria are not allowed during the ongoing study. If there is a clinical indication for any treatment specifically prohibited during the trial, discontinuation from trial therapy may be required. The investigator should discuss any questions regarding this with the Principal Investigator. The final decision on any supportive therapy rests with the investigator and/or the subject's primary physician. However, the decision to continue the subject on trial therapy requires the mutual agreement of the investigator and the subject.

5.6 Rescue Treatments & Supportive Care

No rescue or supportive treatments are specified to be used in this trial.

5.7 Diet/Activity/Other Considerations

Not required.

5.7.1 Diet

Subjects should maintain their usual diet throughout the duration of the trial.

5.7.2 Use of Alcohol, Caffeine, and Tobacco

The site should advise subjects that alcohol should NOT be consumed during the study. The site should advise subjects to limit their alcohol intake as follows:

Refrain from consuming any alcohol for at least 24 hours prior to the study.

The site should advise subjects to limit their caffeine consumption as follows:

Limit their daily caffeine consumption to ≤600 mg caffeine. (See Appendix 12.7 for additional guidance on caffeine products and content.). In addition, caffeinated products should not be consumed until after completion of all procedures at the visit.

The site should advise subjects to limit their tobacco use as follows:

Refrain from the equivalent of >15 cigarettes a day during the study, and

Refrain from smoking during the study

5.7.3 Activity

NA

5.8 Subject Withdrawal/Discontinuation Criteria

Subjects may withdraw consent at any time for any reason or be dropped from the trial at the discretion of the investigator should any untoward effect occur. In addition, a subject may be withdrawn by the investigator if enrollment into the trial is inappropriate, the trial plan is violated, or for administrative and/or other safety reasons. Specific details regarding discontinuation or withdrawal procedures are provided in Section 7.1.4 – Other Procedures.

Table 2 provides reasons why a subject must be discontinued from treatment but may continue to be monitored in the trial, as well as reasons why a subject must be discontinued from treatment and the trial.

Table 2 Discontinuation Scenarios

Reason for Discontinuation Scenario	**Action**
The subject or legal representative (such as a parent or legal guardian) withdraws consent.	Discontinuation from Treatment and Trial
The subject has a medical condition or personal circumstance which, in the opinion of the investigator, places the subject at unnecessary risk through continued participation in the trial or does not allow the subject to adhere to the requirements of the protocol.	Discontinuation from Treatment and Trial
The subject is no longer able to participate in the trial or is not compliant with trial-related procedures.	Discontinuation from Treatment and Trial
The subject takes a prohibited treatment during the trial.	This deviation should be documented and consulted

	regarding the management of the subject.
Subjects who report suicidal ideation with intent, with or without a plan or method (i.e., a positive response to items 4 or 5 in the assessment of suicidal ideation on the C-SSRS) or suicidal behavior may meet discontinuation criteria.	Please refer to Section 7.1.2.2.11, 7.2.3.2 and the ECI guidance document for details.

Discontinuation from treatment is “permanent.” Once a subject is discontinued, he/she shall not be allowed to restart treatment.

5.9 Subject Replacement Strategy

No subject replacement is intended.

5.10 Beginning and End of the Trial

The overall trial begins when the first subject signs the informed consent form. The overall trial ends when the last subject completes the last study-related phone-call or visit, discontinues from the trial, or is lost to follow-up (i.e., the subject is unable to be contacted by the investigator).

5.11 Clinical Criteria for Early Trial Termination

There are no pre-specified criteria for terminating the trial early.

6.0 TRIAL FLOW CHART

Table 3 Trial flow chart

Stage 1 **Visit Number**	**1**	**2**	**3-5**	**6**
Visit Title	**Screen**	**Baseline**	**Stimulation**	**End**
Scheduled Week	0	1	2-3	4
Scheduling Window by Days:	3	3	2	5
Administrative Procedures				
Informed Consent	X			
Inclusion/Exclusion Criteria	X			
Identification Card	X			
Medical History	X			
Prior Medication Review	X			
Monitor Trial Device Compliance		X	X	
Clinical Procedures Assessments				
TPS stimulations	X	X	X	
Vital Signs		X	X	X
Physical Exam		X	X	X
MRC sum score		X	X	X
Hand-held dynamometry		X	X	X
Brain MRI		X		
TMS		X		
Neurological examination	X	X	X	X
C-SSRS	X	X	X	
ALSFRS-R	X	X	X	X
Blood draw		X	X	X
SVC		X		

Stage 2 Visit Number	**1**	**2**	**3-5**	**6-7**	**8**	**9**	**10-16**	**17-18**	**19**
Visit Title	**Screen**	**Rand Base**	**Stim**	**Stim FU**	**End DBP**	**Onset OLE**	**Stim 3**	**Stim FU**	**End OLE**
Scheduled Week	0	1	2-3	8-16	16	16	16-18	26-34	34
Scheduling Window by Days:	3	3	2	2	5	7	2	2	5
Administrative Procedures									
Informed Consent	X								
Inclusion/Exclusion Criteria	X								
Identification Card	X								
Medical History	X								
Prior Medication Review	X								
Monitor Trial Device Compliance		X	X	X			X	X	
Clinical Procedures Assessments									
TPS stimulations	X	X	X	X			X	X	
Vital Signs		X	X	X	X	X	X	X	X
Physical Exam		X	X	X	X	X	X	X	X
MRC sum score		X	X	X	X	X	X	X	X
Hand-held dynamometry		X	X	X	X	X	X	X	X
Brain MRI	X								
TMS	X	X	X	X	X				
Neurological examination	X	X	X	X	X	X	X	X	X
C-SSRS	X	X	X	X			X	X	
ALSFRS-R	X	X	X	X	X	X	X	X	X
Blood draw		X	X	X	X				
SVC		X				X			

Stim: TPS stimulation (6 times in 2 weeks, 20 min each); rand: randomization; Base: baseline; DBP: double blind period; OLE: Open Label Extension

7.0 TRIAL PROCEDURES

7.1 Trial Procedures

The Trial Flow Chart - Section 6.0 summarizes the trial procedures to be performed at each visit. Individual trial procedures are described in detail below. It may be necessary to perform these procedures at unscheduled time points if deemed clinically necessary by the investigator.

7.1.1 Administrative Procedures

7.1.1.1 Informed Consent

The investigator or qualified designee must obtain documented consent from each potential subject or each subject's legally acceptable representative prior to participating in a clinical trial. Documented consent from each subject (referred to as subject consent) will also be obtained by the investigator or qualified designee.

7.1.1.1.1 General Informed Consent

Consent will be documented by the subject's dated signature on a consent form along with the dated signature of the person conducting the consent discussion.

A copy of the respective signed and dated consent forms (subject consents) will be given to the subject and subject before participation in the trial.

The initial subject informed consent forms, any subsequent revised written informed consent forms and any written information provided to the subject will receive the Ethic Committee of the Hospital del Mar approval/favorable opinion in advance of use. The subjects will be informed in a timely manner if new information becomes available that may be relevant to their willingness to continue participation in the trial. The communication of this information will be provided and documented via a revised consent form(s) or addendum to the original consent form(s) that captures the subject's dated signature.

Relevant clinical or MRI findings will be disclosed to the patient and refer for appropriate care.

Specifics about a trial and the trial population will be added to the consent form template(s) at the protocol level. Informed consent(s) will adhere to the ethics Committee requirements, applicable laws and regulations.

7.1.1.2 Inclusion/Exclusion Criteria

If it is determined that the subject does not meet the inclusion/exclusion criteria at visits, the subject will be withdrawn from the study.

7.1.1.3 Subject Identification Card

All subjects will be given a Subject Identification Card identifying them as participants in a research trial. The card will contain trial site (Hospital del Mar) contact information (including direct telephone numbers) to be utilized in the event of an emergency. The investigator or qualified designee will provide the subject with a Subject Identification Card immediately after the subject provides written informed consent.

7.1.1.4 Medical History

A medical history will be obtained by the investigator or qualified designee.

7.1.1.5 Prior and Concomitant Treatments Review

7.1.1.5.1 Prior Treatments

The investigator or qualified designee will review any prior therapeutic drug or neurostimulation use, in advance to visit 2, and record prior treatment taken by the subject within 30 days before starting the trial.

7.1.1.5.2 Concomitant Treatments

The investigator or qualified designee will record treatment(s), if any, taken by the subject during the trial. Any changes to treatment(s) (i.e., dose, frequency) will also be recorded. If the subject reports taking any prohibited treatments during the study, this will be recorded as a study deviation. Concomitant treatments will not be changed during the study, without first consulting the investigator, except in cases of medical emergencies or other obvious exceptions. Please see Section 5.5 for more details.

7.1.1.6 Assignment of Screening Number

All consented subjects will be given a unique screening number that will be used to identify the subject for all procedures that occur prior to randomization or treatment allocation. Each subject will be assigned only one screening number. Screening numbers must not be re-used for different subjects.

7.1.1.7 Assignment of Treatment/Randomization Number

All eligible subjects will be randomly allocated and will receive a treatment/randomization number. The treatment/randomization number identifies the subject for all procedures occurring after treatment allocation/randomization. Once a treatment/randomization number is assigned to a subject, it can never be re-assigned to another subject.

A single subject cannot be assigned more than 1 treatment/randomization numb

7.1.1.8 Trial Compliance (Treatment)

During stimulation visits, administration of TPS therapy will be witnessed by the investigator and/or trial staff. Accounting of trial treatment will be conducted as specified in the Trial Flow Chart.

7.1.1.9 Interactive Voice Response System/Integrated Web Response System

The investigator or designee will call/log into IVRS/IWRS as specified in the Trial Flow Chart. Upon confirmation of a subject's eligibility at Visit 2, the investigator or designee will call IVRS or log into IWRS to randomize the subject. Subjects who do not meet eligibility criteria at Visit 4 will be screen-failed in IVRS/IWRS. For all randomized subjects, the investigator or designee will continue to call/log into IVRS/IWRS as per the Trial Flow Chart. For completed or discontinued subjects, the investigator or designee will make the final call/web action into IVRS/IWRS at their last trial visit. For additional information, please refer to Section 5.3.

7.1.2 Clinical Procedures/Assessments

7.1.2.1 Physical Assessments/Examinations

7.1.2.1.1 Physical Examination/Neurological Examination (PE/Neuro Exam)

A complete physical examination (PE), including a neurological exam, will be performed by a primary investigator or sub-investigator. This examination will also be performed in the event of early discontinuation. The following body systems should be included in these exams:

- Head, Eyes, Ear, Nose, and Throat
- Neck
- Cardiovascular system
- Respiratory system
- Abdomen
- Skin
- Extremities
- Neurological system (see Appendix 12.11)
- Musculoskeletal system

Examination of the breast, rectum, and urogenital system are at the investigator's discretion (i.e., clinically indicated). If a physical or neurological abnormality is noted post-treatment, the investigator will indicate whether the result is clinically significant and if it constitutes an adverse event.

7.1.2.1.3 Vital Signs

Body Temperature: Body temperature will be measured with an oral or tympanic thermometer. The same method (e.g., oral, or tympanic, ºC) should be used for all measurements for each individual subject and should be the same for all subjects throughout the trial.

Heart Rate (HR), Blood Pressure (BP) and Respiratory Rate (RR): Subjects should be resting **for at least 10 minutes** prior to having vital sign measurements obtained. The **same position** should be used for all measurements for each individual subject and should be the same for all subjects throughout the trial. The correct size of the blood pressure cuff and the correct positioning on the subject's arm is essential to increase the accuracy of blood pressure measurements. The same method (e.g., manual, or automated) should be used for all measurements for each individual subject and should be the same for all subjects throughout the study.

7.1.2.1.4 Body Height/Weight

Height (cm/in) and body weight (kg/lbs.) will be collected and recorded. Measurements should be recorded to the nearest centimeter/inch and kilogram/pound. Body weight data will be collected without shoes and with heavy clothing (such as coats) removed. Body weight should be performed on the same scale for the same individual throughout the study.

7.1.2.2 Neurological and Cognitive exam

At baseline and by end of the visit 2 (stimulation) a neurological exam (as defined in section 12.6) will be administered for identifying CNS related adverse events.

7.1.2.2.2 The ALSFRS-R

The ALS Functional Rating Scale in its revised version (ALSFRS-R)[32] is a disease-specific severity score that reflects motor impairment and functional deterioration in people with amyotrophic lateral sclerosis (ALS). It has been widely applied in both clinical practice and ALS research. The scale is disease-specific and encompasses 12 prompts-referred to as items-grouped into four domains to assess bulbar symptoms, limb and trunk functionality, respiratory symptoms, and the need for percutaneous endoscopic gastrostomy, non-invasive ventilation, or tracheostomy with invasive ventilation. The precursor scale was

initially developed as an outcome measure for clinical trials, but over time its revised version became commonly used in both ALS research and clinical practice

7.1.2.2.2.1. MRC sum score

The muscle strength of the limbs will be scored using the Medical Research Council (MRC) sum score, an established multifactorial scoring system with a total score between 0 and 60[33]. The MRC sum score evaluates global muscle strength by combining the scores of six muscles (shoulder abductors, elbow flexors, wrist extensors, hip flexors, knee extensors, and ankle dorsiflexors) that are evaluated bilaterally.

7.1.2.2.2.2 Dynamometry

Hand-held dynamometry megascore (average of z-scores across 16 muscle groups in the arms and legs, with higher values indicating greater strength) will be obtained as described in[34].

7.1.2.2.3 TMS: motor threshold and excitability assessment

Studies using transcranial magnetic stimulation (TMS) have established cortical motoneuron hyperexcitability, heralded by decreased short-interval intracortical inhibition (SICI), as an early feature of sporadic and familial ALS[35]. Greater cortical excitability is prognostic of disease progression. Motor cortex excitability has been used previously as a relevant endpoint in clinical trials in patients with ALS.[36] Paired-pulse transcranial magnetic stimulation (ppTMS) will be conducted in both motor cortex at each side to record: 1) motor evoked potential (MEP) amplitudes; 2) resting motor threshold; 3) intra-cortical facilitation (ICF); and 4) short intracortical inhibition (SICI)[37]. The primary criteria for defining changes in motor excitability related with the therapy is SICI[36]. MEP amplitude was measured at increasing stimulator strengths (eg, 120%, 140%, and 150% of RMT)

7.1.2.2.4 SVC

The slow vital capacity (SVC) is the volume of air expired through an unforced maneuver measured by spirometry. SVC of 50% or greater of estimated measure is required to participate in the study.

7.1.2.2.5. The Columbia-Suicide Severity Rating Scale (C-SSRS)

Suicidal ideation and behavior will be prospectively assessed during this study using the Columbia Suicide Severity Rating Scale (C-SSRS). The C-SSRS will be administered by trained raters at specified time points, as indicated in the Trial Flow Chart, as well as at unscheduled visits as clinically indicated. Site staff will review the contents of the C-SSRS for completeness. Note that the C-SSRS assessment is not considered complete until all available sources of information have been considered (e.g., subject input may be necessary).

The C-SSRS is not explicit about whether the subject specifically has ideation at the time of screening. If a subject reports a prior history of ideation/behavior, the assessor will also inquire and document if this is also present at the time of the screening visit.

Subjects who at any time during this trial report suicidal ideation or behavior that is considered to be an adverse event, either between visits or during visit interviews, must be assessed by the Investigator. Subjects who report suicidal ideation with intent, with or without a plan or method (i.e., a positive response to items 4 or 5 in the assessment of suicidal ideation on the C-SSRS) or suicidal behavior must be evaluated that day by a

psychiatrist or other trained mental health professional who is a licensed psychologist, social worker, or mental health nurse practitioner (or comparable professional qualification in countries outside the United States). Only subjects whose suicidal ideation is passive, who expressly deny any intent to act, and who, after evaluation, are not judged to be at serious risk for self-harm during the trial may continue in the study; others must be discontinued from trial participation and receive appropriate clinical follow-up care to ensure their safety. In addition, any reports of 1) suicidal behavior; 2) suicidal ideation with intent, with or without a plan or method; or 3) suicidal ideation considered to be a clinically significant change, including all cases of suicidal ideation that are treatment- emergent (i.e., start or worsen after trial treatment is initiated) must be recorded as an Event of Clinical Interest (See Section 7.2.3.2). Sites will designate which health care professionals are to be responsible for acute care on-site and to specify referral center(s) to be used for further evaluation. The C-SSRS will be completed in paper form, and will be scored by the qualified trained rater, via a subject interview.

7.1.3 Laboratory Procedures/Assessments

7.1.3.1. Neurofilaments light (NfL) levels in plasma

Plasma NfLs has been validated as outcomes for ALS trials, based on tofersen approval by FDA[34]. At the designed visits, blood will be collected, plasma will be extracted and frozen until its use. Plasma NfL will be measured by Simoa at baseline, after each stimulation session and by end of the DBP and end of the OLE.

7.1.3.2. Biomarkers in nEVs

Extracellular vesicles of neuronal origin (nEVs) is a promising strategy to identify new biomarkers. Such vesicles can be isolated from plasma (even frozen plasma) and reflects the cytoplasm content of the neuronal population 12h in advance. The vesicles content is representative of the cytoplasm and includes proteins, RNAs and metabolites. Then, nEVs can be considered as a surrogate approach to a liquid biopsy of the brain. As such, several biomarkers have been identified in patients with ALS[26], Alzheimer disease[27, 28], Parkinson disease[29, 30], traumatic brain injury or MS[31]. Here, we propose collecting nEVs to conduct a biomarker search study to identify biomarkers associated with the TPS therapy in the motor cortex of people with ALS.

Multiple protein related with the pathogenesis of ALS or CNS damage (SOD1, Ataxin 2, C90rf72, UNC13A, TDP43, stathmin 2, NfL and NfH levels) has been proposed as biomarkers of ALS[39]. However, their levels required a lumbar tap, reducing their feasibility. The use of extracellular vesicles of neuronal origin will allow to determine such proteins reflecting the neurons cytoplasmatic content 12h before[26]. 20 ml of blood will be collected at the designated visits (5 visits = 100 ml in total) and plasma and serum will be collected and stored at -80C until analysis (no DNA will be collected). Protein levels will be measured by SIMOA, MSD or WES at Villoslada's lab.

7.1.4 Other Procedures

7.1.4.1 Withdrawal/Discontinuation

When a subject discontinues/withdraws from participation in the trial during visit 2 (before completing the three planned stimulations), all applicable activities scheduled for the end of visit 2, as outlined in Section 6.0 Trial Flow Chart, will be performed at the time of

discontinuation. Any adverse events which are present at the time of discontinuation/withdrawal will be followed in accordance with the safety requirements outlined in Section 7.2 - Assessing and Recording Adverse Events

7.1.4.2 Blinding/Unblinding

When the investigator or sub-investigator needs to identify the treatment arm used by a subject in case of emergency e.g., the occurrence of serious adverse experiences, he/she will contact the emergency unblinding call center by telephone and make a request for emergency unblinding. As requested by the investigator or sub-investigator the emergency unblinding call center will provide the information to him/her promptly and report unblinding. The emergency unblinding call center will make a record promptly however, the investigator or sub-investigator must enter the intensity of the adverse experiences observed, their relation to study arm, the reason thereof, etc., in the medical chart etc., before unblinding is performed. Additionally, the investigator must go into the IVRS system and perform the unblind in the IVRS system to update arm disposition. If the emergency unblinding call center is not available for a given site in this trial, IVRS/IWRS should be used for emergency unblinding in the event that this is required for subject safety.

If unblinding has occurred, the circumstances around the unblinding (e.g., date and reason) must be documented promptly, and principal investigator notified as soon as possible. Only the principal investigator or delegate and the respective subject's code should be unblinded. Trial site personnel directly associated with the conduct of the trial should not be unblinded.

7.1.4.3 Domiciling

NA

7.1.4.4 Calibration of Critical Equipment

TPS device is calibrated as follows: the electromagnetic stimulator is calibrated by the vendor (Storz medical) every 2 million pulses. The distillated water of the stimulator is replaced every 6 months. The systems have no other calibration or maintenance requirements.

7.1.5 Visit Requirements

Visit requirements are outlined in Section 6.0 - Trial Flow Chart. Specific procedure-related details are provided above in Section 7.1 - Trial Procedures.

7.1.5.1 Visits

Each visit should be performed as noted in the Trial Flow Chart. For visits that require additional explanations, please see those specific visits below.

7.1.5.1.1 Visit 1: Screening, brain MRI, TMS and SVC

At Visit 1, subjects who provide informed consent will undergo a series of diagnostic and safety assessments to determine if they are eligible for the trial. A designated subject must also consent to participate and will be asked to complete certain assessments throughout the trial. Subjects planning to undergo elective procedures during the study (known prior to trial start) should not proceed until such procedures have been completed.

At the screening visit, medical history, use of drugs, alcohol, caffeine, smoking will be recorded. In addition urine test and the Columbia-Suicide Severity Rating Scale (C-SSRS) will be administered. The SVC will be collected by spirometry.

If the subject fulfills the inclusion criteria and signs the informed consent, a brain MRI and a TMS study will be scheduled in the following 2-7 days (always before Visit 2). Once obtained the brain MRI, it will be uploaded to the TPS device for neuronavigation purposes. TMS will be conducted for defining presence of hyper-excitability.

7.1.5.1.2 Visit 2: baseline

Patients will be randomized to either treatment or sham stimulation and assigned a code (except for patient sin stage 1, which are not randomized, and all receive active stimulation). Before starting stimulation, the researcher will conduct a vital signs, physical exam and neurological examination. Urine (50 ml) and blood (20 ml) will be collected and stored. Then, the patient will be subjected to the first TPS stimulations (a 20 min stimulations).

7.1.5.1.3 Visit 3-5, 6-12, 15-21 and 22-28: stimulation visits

At each visit the patient will be reassessed for vital signs, physical exam and neurological exam. After the completion of each 6-session period, the C-SSRS will be administered. Blood and urine will be collected the last day of stimulation for each 6-session period. TMS and ALSFRS-R will be assessed the last day of stimulation for each 6-session period.

7.1.5.1.2 Visit 13, 14 and 29: end of stage 1, end of DBP, onset and end of OLE of stage 2

During such visits, vital signs, physical exam, and neurological exam and ALSFRS-R will be conducted.

7.2 Assessing and Recording Adverse Events

An adverse event is defined as any untoward medical occurrence in a clinical investigation subject administered a medical device treatment and which does not necessarily have to have a causal relationship with this treatment. An adverse event can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product or protocol specified procedure, whether considered related to the medicinal product or protocol specified procedure. Any worsening (i.e., any clinically significant adverse change in frequency and/or intensity) of a preexisting condition that is temporally associated with the use of the product, is also an adverse event.

Changes resulting from normal growth and development that do not vary significantly in frequency or severity from expected levels are not to be considered adverse events. Examples of this may include, but are not limited to, teething, typical crying in infants and children and onset of menses or menopause occurring at a physiologically appropriate time.

Product includes any device, diagnostic agent, pharmaceutical product, biological product, or protocol-specified procedure, whether investigational (including sham or active comparator treatment) or marketed, manufactured by, licensed by, provided by, or distributed by the vendor for human use.

Adverse events may occur during clinical trials, or as prescribed in clinical practice, from inadequate therapeutic regimen (whether accidental or intentional), from abuse and from withdrawal.

Electronic reporting procedures can be found in the electronic data capture (EDC) entry guidelines. Paper reporting procedures can be found in the Investigator Trial File Binder (or equivalent).

7.2.1 Definition of an Over-treatment for this Protocol and Reporting of Over-treatment

In this trial, an over-treatment is any therapeutic regimen of higher intensity or of longer duration than the specified therapeutic regimen to be administered in a calendar day (accidental or intentional). If an adverse event(s) is associated with ("results from") the over-treatment, the adverse event(s) is reported as a serious adverse event, even if no other seriousness criteria are met. If the therapeutic regimen meeting the protocol definition of over-treatment is taken without any associated clinical symptoms or abnormal laboratory results, the over-treatment is reported as a non-serious Event of Clinical Interest (ECI), using the terminology "accidental or intentional over-treatment without adverse effect."

All reports of over-treatment with and without an adverse event must be reported by the investigator within 24 hours either by electronic media or paper. Electronic reporting procedures can be found in the electronic data capturing (EDC) entry guidelines. Paper reporting procedures can be found in the Investigator Trial File Binder (or equivalent).

7.2.2 Reporting of Pregnancy and Lactation

Female participants may screen for this study if:

- They are surgically sterile [have had a hysterectomy (uterus removed), bilateral oophorectomy (ovaries removed), or tubal ligation at least 6 months prior]
- They are of post-menopausal age and have not had a menstrual period for 12 months
- They have a vasectomized partner (performed at least 6 months prior) who has been documented to no longer produce sperm
- They are using a highly effective method of contraception to avoid pregnancy throughout the study and for 30 days after you complete this study.

Examples of acceptable forms of highly effective contraception include:

1. Established use of oral, injected or implanted hormonal methods of contraception plus use of a condom for your male partner.
2. Placement of an intrauterine device (IUD) or intrauterine system (IUS) plus use of a condom for your male partner.
3. True abstinence: When this is in line with your preferred and usual lifestyle

NOTE: Periodic abstinence (e.g., calendar, ovulation, symptothermal, post ovulation methods), condoms alone or double barrier are not acceptable methods of contraception.

Male participants must ensure a condom is used for all sexual intercourse as well as following the acceptable methods of contraception listed above for your female partner and ensuring that they are used for the entire duration of the study, and for at least 90 days after you complete this study.

Although pregnancy and lactation are not considered adverse events, it is the responsibility of investigators or their designees to report any pregnancy or lactation in a subject (spontaneously reported to them) that occurs during the trial.

7.2.3 Immediate Reporting of Adverse Events

7.2.3.1 Serious Adverse Events

A serious adverse event is any adverse event occurring at any therapeutic regimen or during any use of the product that:

- Results in death;
- Is life threatening;
- Results in persistent or significant disability/incapacity;
- Results in or prolongs an existing inpatient hospitalization;
- Is a congenital anomaly/birth defect;
- Is another important medical event.

Note: In addition to the above criteria, adverse events meeting either of the below criteria, although not serious per ICH definition, are reportable in the same timeframe as SAEs to meet certain local requirements. Therefore, these events are considered serious for collection purposes.

- Is a cancer;
- Is associated with an overdose.

Refer to Table 6 for additional details regarding each of the above criteria.

For the time period beginning when the consent form is signed until treatment, any serious adverse event, or follow up to a serious adverse event, including death due to any cause, that occurs to any subject must be reported within 24 hours for weekly days or 72 days over the weekend if it causes the subject to be excluded from the trial, or is the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, sham treatment or a procedure. The 24 hours for the site starts when the site becomes aware of the SAE. SAEs not reported will be considered a major protocol deviation.

For the time beginning at treatment allocation through 14 days following cessation of treatment, any serious adverse event, or follow up to a serious adverse event, including death due to any cause, whether or not related to the product, must be reported within 24 hours either by electronic media or paper. Electronic reporting procedures can be found in the EDC data entry guidelines. Paper reporting procedures can be found in the Investigator Trial File Binder (or equivalent).

Additionally, any serious adverse event, considered by an investigator who is a qualified physician to be related to the product that is brought to the attention of the investigator at any time outside of the time specified in the previous paragraph also must be reported immediately.

All subjects with serious adverse events must be followed up for outcome.

7.2.3.2. Events of Clinical Interest (during the stimulation visit 2 (in the 1h after each stimulation)

1. Agitation – daytime or nighttime
2. Confusion or cognitive impairment - daytime or nighttime

Regarding items #1 and 2 above, agitation, confusion or cognitive impairment should be considered an ECI if in the investigator's opinion an acute worsening from baseline has occurred, or there is an unusual or atypical presentation of symptoms for a given subject.

7.2.3.3 Protocol-Specific Exceptions to Serious Adverse Event Reporting

7.2.4 Evaluating Adverse Events

An investigator who is a qualified physician will evaluate all adverse events with respect to the elements outlined in Table 4. The investigator's assessment of causality is required for each adverse event. Refer to Table 4 for instructions in evaluating adverse events.

Table 4 Evaluating Adverse Events

Maximum Intensity	**Mild**	awareness of sign or symptom, but easily tolerated (for pediatric trials, awareness of symptom, but easily tolerated)
	Moderate	discomfort enough to cause interference with usual activity (for pediatric trials, definitely acting like something is wrong)
	Severe	incapacitating with inability to work or do usual activity (extremely distressed or unable to do usual activities)
Seriousness	A serious adverse event (SAE) is any adverse event occurring at any Therapeutic regimen or during any use of product that:	
	†**Results in death**; or	
	†**Is life threatening; or** places the subject, in the view of the investigator, at immediate risk of death from the event as it occurred [Note: This does not include an adverse event that, had it occurred in a more severe form, might have caused death.]; or	
	†**Results in a persistent or significant disability/incapacity** (substantial disruption of one's ability to conduct normal life functions); or	
	†**Results in or prolongs an existing inpatient hospitalization** (hospitalization is defined as an inpatient admission, regardless of length of stay, even if the hospitalization is a precautionary measure for continued observation. (Note: Hospitalization for an elective procedure to treat a pre-existing condition that has not worsened is not a serious adverse event. A pre-existing condition is a clinical condition that is diagnosed prior to the use of a Hospital del Mar neurosciences product and is documented in the patient's medical history.); or	
	†**Is a congenital anomaly/birth defect (**in offspring of subject taking the product regardless of time to diagnosis); or	
	Is a cancer (although not serious per ICH definition, is reportable within 24 hours to meet certain local requirements); **or**	
	Is associated with an over-treatment (whether accidental or intentional). Any adverse event associated with an over-treatment is considered a serious adverse event for collection purposes. An over-treatment that is not	

	associated with an adverse event is considered a non-serious event of clinical interest and must be reported within 24 hours.	
	Other important medical events that may not result in death, not be life threatening, or not require hospitalization may be considered a serious adverse event when, based upon appropriate medical judgment, the event may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed previously (designated above by a †).	
Duration	Record the start and stop dates of the adverse event. If less than 1 day, indicate the appropriate length of time and units	
Action Taken	Did the adverse event cause the product to be discontinued?	
Relationship to Product	Did the product cause the adverse event? The determination of the likelihood that the product caused the adverse event will be provided by an investigator who is a qualified physician. The investigator's signed/dated initials on the source document or worksheet that supports the causality noted on the AE form, ensures that a medically qualified assessment of causality was done. This initialed document must be retained for the required regulatory time frame. The criteria below are intended as reference guidelines to assist the investigator in assessing the likelihood of a relationship between the test drug and the adverse event based upon the available information **The following components are to be used to assess the relationship between the product and the AE**; the greater the correlation with the components and their respective elements (in number and/or intensity), the more likely the p product caused the adverse event:	
	Exposure	Is there evidence that the subject was actually exposed to the product such as: reliable history, acceptable compliance assessment (pill count, diary, etc.), expected pharmacological effect, or measurement of drug/metabolite in bodily specimen?
	Time Course	Did the AE follow in a reasonable temporal sequence from administration of the product? Is the time of onset of the AE compatible with a drug-induced effect (applies to trials with investigational medicinal products)?
	Likely Cause	Is the AE not reasonably explained by another etiology such as underlying disease, other drug(s)/vaccine(s), or other host or environmental factors

Relationship to the Product (continued)	**The following components are to be used to assess the relationship between the product and the AE (continued)**	
	Dechallenge	Was the product discontinued or dose/exposure/frequency reduced? If yes, did the AE resolve or improve? If yes, this is a positive dechallenge. If not, this is a negative dechallenge. (Note: This criterion is not applicable if: (1) the AE resulted in death or permanent disability; (2) the AE resolved/improved despite continuation of the product; (3) the trial is a single-Therapeutic regimen drug trial); or (4) product(s) is/are only used one time.)
	Rechallenge	Was the subject re-exposed to the product in this trial? If yes, did the AE recur or worsen? If yes, this is a positive rechallenge. If not, this is a negative rechallenge. (Note: This criterion is not applicable if: (1) the initial AE resulted in death or permanent disability, or (2) the trial is a single-Therapeutic regimen drug trial); or (3) product(s) is/are used only one time.) NOTE: IF A RECHALLENGE IS PLANNED FOR AN ADVERSE EVENT WHICH WAS SERIOUS AND WHICH MAY HAVE BEEN CAUSED BY THE PRODUCT, OR IF RE-EXPOSURE TO THE PRODUCT POSES ADDITIONAL POTENTIAL SIGNIFICANT RISK TO THE SUBJECT THEN THE RECHALLENGE MUST BE APPROVED IN ADVANCE BY THE CLINICAL DIRECTOR AND THE INSTITUTIONAL REVIEW BOARD/INDEPENDENT ETHICS COMMITTEE.
	Consistency with Trial Treatment Profile	Is the clinical/pathological presentation of the AE consistent with previous knowledge regarding the product or drug class pharmacology or toxicology?
The assessment of the relationship will be reported on the case report forms /worksheets by an investigator who is a qualified physician according to his/her best clinical judgment, including consideration of the above elements.		
Record one of the following:	**Use the following scale of criteria as guidance (not all criteria must be present to be indicative of a**	

	product relationship).
Yes, there is a reasonable possibility of product relationship.	There is evidence of exposure to the product. The temporal sequence of the AE onset relative to the administration of the product is reasonable. The AE is more likely explained by the product than by another cause.
No, there is not a reasonable possibility of product relationship	Subject did not receive the product OR temporal sequence of the AE onset relative to administration of the product is not reasonable OR the AE is more likely explained by another cause than the product. (Also entered for a subject with over-treatment without an associated AE.)

7.2.5 Investigator Responsibility for Reporting Adverse Events

All Adverse Events will be reported to the Ethical Committee of Hospital del Mar and investigators in accordance with all applicable global laws and regulations, i.e., per ICH Topic E6 (R1) Guidelines for Good Clinical Practice.

8.0 STATISTICAL ANALYSIS PLAN

8.1 Statistical Analysis Plan Summary

Considering this is the first study in a very small population for assessing the safety and tolerability and getting insights on the efficacy, results will be provided only as a descriptive statistical analysis

Table 5 Statistical analysis plan

Study Design Overview	Modulation of the motor pathway by Transcranial Pulse Stimulation in people with ALS: a pilot randomized trial
Treatment Assignment	Active stimulation: 36,000 pulses in 2 weeks distributed in 6 sessions Sham: stimulations using a sound blocker (membrane filled with air)
Analysis Populations	Intention to treat Per Protocol
Primary Endpoint(s)	Stage 1. Change in the motor cortex excitability threshold from baseline to week 8. Stage 2. Change from baseline to month 6 in the ALS functional rating scale-revised (ALSFRS-R) total score.
Key Secondary Endpoints	Secondary stage 1: 1. Change in the motor cortex excitability (paired-pulse transcranial magnetic stimulation (ppTMS): motor evoked potential (MEP) amplitudes, resting motor threshold, intra-cortical facilitation (ICF) and short intracortical inhibition (SICI)), from baseline to week 4 2. Change plasma NfL levels from baseline to week 4 and 8 3. Changes in muscle strength, assessed by MRC sum score from baseline to week 4 and 8 4. Changes in hand-held dynamometry from baseline to week 4 and 8Secondary stage 2: 1. Differences (treated vs sham) and change (from baseline to end of the study (month 6)) in the slow vital capacity (SVC) 2. Differences (treated vs sham) and change (from baseline to end of the study (month 6)) in plasma NfL levels or other biomarkers from exploratory stage 1 if found significant

	3. Differences (treated vs sham) and change (from baseline to end of the study (month 6)) in in cortical hyperexcitability (MEP amplitudes, resting motor threshold, ICF and SICI) 4. Changes in muscle strength, assessed by MRC sum score from baseline to end of the study (month 6) 5. Changes in hand-held dynamometry, from baseline to end of the study (month 6)
Exploratory endpoints	<u>Exploratory stage 1</u> 1. Changes in SOD1, Ataxin 2, C90rf72, UNC13A, TDP43, NfL and NfH levels in extracellular vesicles of neuronal origin (nEVs) collected from plasma from baseline to week 4 and 8 <u>Exploratory stage 2</u> 1. Differences (treated vs sham) and change (from baseline to end of the study (month 6)) in biomarker levels in nEVs collected from plasma that were informative in stage 1 from baseline to week 4 and 8
Statistical Methods for Key Efficacy Analyses	Changes on ALSFRS-R, motor threshold, biomarker levels before and after stimulation will be assessed with ANCOVA test.
Statistical Methods for Key Safety Analyses	Descriptive statistics
Interim Analyses	No interim analysis for efficacy or safety is planned for this study.
Multiplicity	Not planned
Sample Size and Power	Considering that this is a pilot study, and no previous data is available for this intervention, a convenience sample size of 10 subjects for and 40 subjects for stage 2 has been defined. Using the results from the CENTAUR trial[1] in patients with ALS, a sample size of 6 patients per arm provided a 90% to detect differences by week 24 with alpha 0.05.

8.2 Responsibility for Analyses

The statistical analysis of the data obtained from this study will be the responsibility of the Principal Investigator.

8.3 Hypotheses

Objectives and hypotheses of the study are stated in Section 3.

8.4 Analysis Endpoints

Key efficacy and safety endpoints that will be evaluated for within- and/or between-TPS protocols are listed below, followed by the descriptions of the derivations of selected endpoints.

8.4.1. Efficacy Endpoints

Rationale for the key efficacy endpoints is given in Section 4.2.3.1 and an initial description of the efficacy measures is included in Section 7.1.2.2. In general, if a baseline value exists for a particular efficacy measure, then the change from baseline in that value will be evaluated.

Primary Endpoint

Stage 1. Change in the motor cortex excitability threshold from baseline to week 8.
Stage 2. Change from baseline to month 6 in the ALS functional rating scale-revised (ALSFRS-R) total score.

Secondary Endpoint

Secondary stage 1:
1. Change in the motor cortex excitability (paired-pulse transcranial magnetic stimulation (ppTMS): motor evoked potential (MEP) amplitudes, resting motor threshold, intra-cortical facilitation (ICF) and short intracortical inhibition (SICI)), from baseline to week 4
2. Change plasma NfL levels from baseline to week 4 and 8
3. Changes in muscle strength, assessed by MRC sum score from baseline to week 4 and 8
4. Changes in hand-held dynamometry from baseline to week 4 and 8Secondary stage 2:
1. Differences (treated vs sham) and change (from baseline to end of the study (month 6)) in the slow vital capacity (SVC)
2. Differences (treated vs sham) and change (from baseline to end of the study (month 6)) in plasma NfL levels or other biomarkers from exploratory stage 1 if found significant
3. Differences (treated vs sham) and change (from baseline to end of the study (month 6)) in in cortical hyperexcitability (MEP amplitudes, resting motor threshold, ICF and SICI)
4. Changes in muscle strength, assessed by MRC sum score from baseline to end of the study (month 6)
5. Changes in hand-held dynamometry, from baseline to end of the study (month 6)

8.4.2 Safety Endpoints

Presence of serious adverse events (together with items of special attention)

An initial description of the safety measures is included in Sections 7.1.2.1, 7.1.2.2.10 and 7.1.3. Safety and tolerability will be assessed by statistical and clinical review of the following data collected throughout the study: adverse experiences (AEs), and treatment-emergent suicidality. The Columbia-Suicide Severity Rating Scale (C-SSRS) will be used to ascertain and document the occurrence of suicidal events (i.e., ideation and behavior) systematically and prospectively. Responses on the C-SSRS are classified according to 11 pre-specified categories as described in Appendix 12.14.

The primary time for safety analyses is Visit 2; Safety endpoints are classified into 2 tiers (see Statistical Methods for Key Safety Analyses in Section 8.1 for Tier definitions).

Tier 1 Safety Endpoints include (the proportion of subjects with):

1. Any AE
2. Any Serious AE
3. Any treatment-Related AE

4. Any Serious and treatment-Related AE

5. Discontinuation due to AE

Tier 2 Safety Endpoints include:

1. Specific AEs, SOC AEs or PDLCs which have incidence in all 3 subjects.

8.5 Analysis Populations

8.5.1 Safety Analysis Population

The safety population will be used for the analysis of safety data in this study. The safety population consists of all subjects who received at least 1 stimulation protocol of trial treatment. Subjects will be included in the treatment group corresponding to the trial treatment they received for the analysis of safety data using the safety population. Subjects who take incorrect trial treatment for the entire treatment period will be included in the treatment group corresponding to the trial treatment received.

At least 1 vital sign measurement obtained after at least 1 stimulation protocol of trial treatment is required for inclusion in the analysis of each specific parameter. To assess change from baseline, a baseline measurement is also required.

8.6 Statistical Methods

Safety and tolerability will be assessed by statistical and clinical review of the following data collected throughout the study: adverse experiences (AEs), and treatment-emergent suicidality derived from the C-SSRS. Safety will be evaluated with doses combined; selected safety analyses will be performed for doses separately.

The analysis of safety results will follow a tiered approach (Table 6). The tiers differ with respect to the analyses that will be performed. Safety parameters or adverse experiences of special interest that are identified *a priori* constitute "Tier 1" safety endpoints that will be subject to inferential testing for statistical significance with p-values and 95% confidence intervals provided for between-group comparisons. Other safety parameters will be considered as Tier 2 or Tier 3. Tier 2 parameters will be assessed via point estimates with 95% confidence intervals provided for between-group comparisons.

Membership in Tier 1 requires that at least 4 subjects in any treatment group exhibit the event; all other adverse experiences and predefined limits of change will belong to Tier 2.

Summary statistics for baseline, on-treatment, and change from baseline values will be provided by the treatment group in table format.

See Table 6 for a classification of safety endpoints as Tier 1, or 2 and the corresponding analysis strategy for each endpoint.

Table 6 Analysis Strategy for Safety Parameters

Safety Tier	Safety Endpoint[†]	p-Value[§]	95% CI for Treatment Comparison[§]	Descriptive Statistics
Tier 1	Any AE		X	X

	Any Serious AE		X	X
	Any Drug-Related AE		X	X
	Any Serious and Drug-Related AE		X	X
	Discontinuation due to AE		X	X
	Specific AEs, SOCs, or PDLCs (incidence ≥4 subjects in 1 of the treatment groups)		X	X
Tier 2	Specific AEs, SOCs or PDLCs [‡] (incidence <4 subjects in all the treatment groups)			X
	Change from Baseline Results (ECGs, Vital Signs)			X

[2] Adverse Event (AE) references refer to both Clinical and Laboratory AEs.

[3] Includes only those endpoints not pre-specified as Tier 1 or not already pre-specified as Tier-2 endpoint

[4] P-value and CI for safety endpoints based upon Miettinen & Nurminen method

Note: SOC=System Organ Class; PDLC=Pre-Defined Limit of Change; X = results will be provided.

8.6.3 Summaries of Baseline Characteristics, Demographics, and Other Analyses

8.6.3.1 Demographic and Baseline Characteristics

Demographic variables (e.g., age, gender, race), baseline characteristics, primary and secondary diagnoses, and prior and concomitant therapies will be summarized by treatment either by descriptive statistics or categorical tables.

8.7 Interim Analysis

No interim analyses for efficacy or safety are planned for this study

8.8 Multiplicity

Not planned.

8.9 Sample Size and Power Calculations

Sample size

This study will recruit 10 patients for stage 1 (open label) and 40 patients for stage 2 (randomized DBP). Using the results from the CENTAUR trial[1] in patients with ALS, a sample size of 6 patients per arm provided a 90% to detect differences by week 24 with alpha 0.05.

Power for the primary hypothesis

Considering that this is a pilot study, and no previous data is available for this intervention, a convenience sample size of 10 subjects for stage 1 and 40 for stage 2 has been defined. Using the results from the CENTAUR trial[1] in patients with ALS, a sample size of 6 patients per arm provided a 90% to detect differences by week 24 with alpha 0.05.

8.10 Subgroup Analyses

NA

8.11 Compliance (Treatment Adherence)

Summary statistics will be provided on percent compliance by treatment for all subjects included.

8.12 Extent of Exposure

The total number of days each subject received a particular total daily stimulation protocol will be identified and then summarized (as subject counts and percentages)

9.0 LABELING, PACKAGING, STORAGE AND RETURN OF CLINICAL SUPPLIES

No drugs are being tested in this study

10.0 ADMINISTRATIVE AND REGULATORY DETAILS

10.1 Confidentiality

10.1.1 Confidentiality of Data

By signing this protocol, the investigator affirms that information furnished to the investigator will be maintained in confidence, and such information will be divulged to the institutional review board, ethics review committee (IRB/ERC) or similar or expert committee, affiliated institution, and employees, only under an appropriate understanding of confidentiality with such board or committee, affiliated institution, and employees. Data generated by this trial will be considered confidential by the investigator, except to the extent that it is included in a publication as provided in the Publications section of this protocol.

10.1.2 Confidentiality of Subject Records

By signing this protocol, the investigator agrees that the Ethical Committee, or regulatory authority representatives may consult and/or copy trial documents to verify worksheet/case report form data. By signing the consent form, the subject agrees to this process. If trial documents will be photocopied during the process of verifying worksheet/case report form information, the subject will be identified by unique code only; full names/initials will be masked prior to transmission.

By signing this protocol, the investigator agrees to treat all subject data used and disclosed in connection with this trial in accordance with all applicable privacy laws, rules, and regulations.

10.1.3 Confidentiality of Investigator Information

By signing this protocol, the investigator recognizes that certain personal identifying information with respect to the investigator, and all sub-investigators and trial site personnel, may be used and disclosed for trial management purposes, as part of a regulatory submissions, and as required by law. This information may include:

1. name, address, telephone number and e-mail address;
2. hospital or clinic address and telephone number;
3. curriculum vitae or other summary of qualifications and credentials; and
4. other professional documentation.

Additionally, the investigator's name and business contact information may be included when reporting certain serious adverse events to regulatory authorities or to other investigators. By signing this protocol, the investigator expressly consents to these uses and disclosures.

10.1.4 Confidentiality of Ethics Committee Information

The principal investigator is required to record the name and address of the Ethics Committee that reviews and approves this trial.

10.2 Compliance with Financial Disclosure Requirements

The investigator/subinvestigator(s) agree to provide his/her financial interests to allow for the submission of complete and accurate certification and disclosure statements. The investigator/subinvestigator(s) further agree to provide this information on a Certification/Disclosure Form, commonly known as a financial disclosure form.

10.3 Compliance with Law, Audit and Debarment

By signing this protocol, the investigator agrees to conduct the trial in an efficient and diligent manner and in conformance with this protocol; generally accepted standards of Good Clinical Practice (e.g., International Conference on Harmonization (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use Good Clinical Practice: all applicable federal, state, and local laws, rules and regulations relating to the conduct of the clinical trial.

The Code of Conduct, a collection of goals and considerations that govern the ethical and scientific conduct of clinical investigations, is provided in Section 12.1 Hospital del Mar Neurosciences Code of Conduct for Clinical Trials.

The investigator also agrees to allow monitoring, audits, Ethics Committee review and regulatory authority inspection of trial-related documents and procedures and provide for direct access to all trial-related source data and documents.

The investigator agrees not to seek reimbursement from subjects, their insurance providers or from government programs for procedures included as part of the trial reimbursed to the investigator.

The investigator shall prepare and maintain complete and accurate trial documentation in compliance with Good Clinical Practice standards and applicable federal, state, and local laws, rules, and regulations; and, for each subject participating in the trial, provide all data, and, upon completion or termination of the clinical trial, submit any other reports to the regulatory agencies.

Trial documentation will be promptly and fully disclosed by the investigator upon request and shall be made available at the trial site upon request for inspection, copying, review and audit at reasonable times from any regulatory authorities. The investigator agrees to promptly take any reasonable steps that are requested because of an audit to cure deficiencies in the trial documentation and worksheets/case report forms.

The investigator must maintain copies of all documentation and records relating to the conduct of the trial in compliance with all applicable legal and regulatory requirements. This documentation includes, but is not limited to, the protocol, worksheets/case report forms, advertising for subject participation, adverse event reports, subject source data,

correspondence with regulatory authorities, consent forms, investigator's curricula vitae, monitor visit logs, laboratory reference ranges, laboratory certification or quality control procedures and laboratory director curriculum vitae. By signing this protocol, the investigator agrees that documentation shall be retained until at least 2 years after the last approval of a marketing application in an ICH region or until there are no pending or contemplated marketing applications in an ICH region or until at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. Because the clinical development and marketing application process is variable, it is anticipated that the retention period can be up to 15 years or longer after protocol database lock. All trial documents shall be made available if required by relevant regulatory authorities. The investigator must consult with and obtain written approval prior to destroying trial and/or subject files.

ICH Good Clinical Practice guidelines recommend that the investigator inform the subject's primary physician about the subject's participation in the trial if the subject has a primary physician and if the subject agrees to the primary physician being informed.

10.4 Compliance with Trial Registration and Results Posting Requirements

The principal investigator of the trial is solely responsible for determining whether the trial and its results are subject to the requirements for submission to EUDRA (http://eudragmdp.ema.europa.eu) or ClinicalTrials.org (http://www.clinicaltrials.gov) or other local registries. Hospital del Mar researchers will review this protocol and submit the information necessary to fulfill these requirements. Information posted will allow subjects to identify potentially appropriate trials for their disease conditions and pursue participation by calling a central contact number for further information on appropriate trial locations and trial site contact information.

By signing this protocol, the investigator acknowledges that the statutory obligations under EMA clinical trials directive or other locally mandated registries are that of the investigators and agrees not to submit any information about this trial or its results to those registries.

10.5 Quality Management System

By signing this protocol, the principal investigator agrees to be responsible for implementing and maintaining a quality management system with written development procedures and functional area standard operating procedures (SOPs) to ensure that trials are conducted and data are generated, documented, and reported in compliance with the protocol, accepted standards of Good Clinical Practice, and all applicable federal, state, and local laws, rules and regulations relating to the conduct of the clinical trial.

10.6 Data Management

The investigator or qualified designee is responsible for recording and verifying the accuracy of subject data. By signing this protocol, the investigator acknowledges that his/her electronic signature is the legally binding equivalent of a written signature. By entering his/her electronic signature, the investigator confirms that all recorded data have been verified as accurate. Detailed information regarding Data Management procedures for this protocol will be provided separately.

10.7 Publications

This trial may be intended for publication, even if terminated prematurely. Publication may include any or all the following: posting of a synopsis online, abstract and/or presentation at a scientific conference, or publication of a full manuscript. The principal investigator will work with the authors to submit a manuscript describing trial results within 12 months after the last data become available, which may take up to several months after the last subject visit in some cases such as vaccine trials. Hospital del Mar researchers will post a synopsis of trial results for approved products on EUDRA or Clinicaltrials.gov by 12 months after the last subject's last visit for the primary outcome, 12 months after the decision to discontinue development, or product marketing (dispensed, administered, delivered, or promoted), whichever is later.

These timelines may be extended for products that are not yet marketed, if additional time is needed for analysis, to protect intellectual property, or to comply with confidentiality agreements with other parties. Authors of the primary results manuscript will be provided the complete results from the Clinical Study Report, subject to the confidentiality agreement. When a manuscript is submitted to a biomedical journal, the policy is to also include the protocol and statistical analysis plan to facilitate the peer and editorial review of the manuscript. If the manuscript is subsequently accepted for publication, the researchers will allow the journal, if it so desires, to post on its website the key sections of the protocol that are relevant to evaluating the trial, specifically those sections describing the trial objectives and hypotheses, the subject inclusion and exclusion criteria, the trial design and procedures, the efficacy and safety measures, the statistical analysis plan, and any amendments relating to those sections.

Authorship credit should be based on 1) substantial contributions to conception and design, or acquisition of data, or analysis and interpretation of data; 2) drafting the article or revising it critically for important intellectual content; and 3) final approval of the version to be published. Authors must meet conditions 1, 2 and 3. Significant contributions to trial execution may also be considered to determine authorship if contributions have also been made to all three of the preceding authorship criteria. Although publication planning may begin before conducting the trial, final decisions on authorship and the order of authors' names will be made based on participation and actual contributions to the trial and writing, as discussed above. The first author is responsible for defending the integrity of the data, method(s) of data analysis and the scientific content of the manuscript.

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12. APPENDICES

12.1. List of Abbreviations and Definition of Terms

AE	Adverse Experience
ASaT	All Subjects as Treated
βhCG	Beta-Human Chorionic Gonadotropin
BMI	Body Mass Index
BP	Blood Pressure
CI	Confidence Interval
CNS	Central Nervous System
CSR	Clinical Study Report
C-SSRS	Columbia-Suicide Severity Scale
DEGs	Data Entry Guidelines
ALS	Amyotrophic Lateral Sclerosis
ECI	Events of Clinical Interest
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture
FSH	Follicle-stimulating Hormone
TPS	Transcranial Pulse Stimulation
GCP	Good Clinical Practice
HR	Heart Rate

IA	Interim Analysis
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonization
IEC	Independent Ethics Review Committee
IRB	Institutional Review Board
kg	Kilogram
NCS	Not Clinically Significant
PDLC	Pre-Defined Limit of Change.
PE	Physical Examination

SAC	Scientific Advisory Committee
SAE	Serious Adverse Experience
SAP	Statistical Analysis Plan
SD	Standard Deviation
SES	Standardized Effect Size
SOC	System Organ Class
SOP	Standard Operating Procedure
SAP	Statistical Analysis Plan
ULN	Upper Limit of Normal

12.2. ALS Diagnosis criteria[5]

The diagnosis of ALS requires: (A) the presence of:

(A: 1) evidence of lower motor neuron (LMN) degeneration by clinical, electrophysiological or neuropathologic examination,

(A:2)evidence of upper motoneuron (UMN) degeneration by clinical examination, and

(A:3)progressive spread of symptoms or signs within a region or to other regions, as determined by history or examination,together with:

(B) the absence of

(B:1) electrophysiological or pathological evidence of other disease processes that might explain the signs of LMN and/or UMN degeneration, and

(B:2) neuroimaging evidence of other disease processes that might explain the observed clinical and electrophysiological signs.

12.3 General Neurological Exam

The General Neurological Examination will be performed at the timepoint(s) specified in the protocol flow chart.

Note to the investigator: If at any time abnormalities are observed in the General Neurological Exam, the Investigator should do additional examinations as needed based on his or her medical judgment.

The **General Neurological Examination** includes all of the modules listed below, ***with the exception of Module 1,*** and is intended to be a general screening examination and sufficient for this study and subject population.

MODULE 2 – CRANIAL NERVE ASSESSMENT

A. II – Visual Fields and acuity
B. II, III – Pupil Size and Reactivity
C. III, IV, VI – Extraocular Movements (range of motion, smooth pursuit, saccades, nystagmus)
    1. Observe for nystagmus during eye movements, increased nystagmus at the end of gaze or other oculomotor changes (mild nystagmus at extremes of gaze is normal). Note direction of nystagmus
D. V – Facial Sensation, Jaw Strength
E. VII – Muscles of Facial Expression (wrinkle brow, squeeze eyes shut, smile)
F. VIII – Auditory Acuity (assessed using a bed-side screening test eg by rubbing fingers on each side of subject's head or by whispering numbers)
G. IX – Gag reflex
H. X – Swallow
I. XI – Shoulder shrug
J. Tongue Protrusion (midline)

Score: left and right (except for G, H, J)

Grade: **NORMAL or IMPAIRED and describe abnormality**

MODULE 3 - MOTOR SYSTEM

A. Muscle Tone
    1. Ask the volunteer to relax.
    2. Flex and extend the volunteer's elbows and knees (bilaterally).
    3. There is a small, continuous resistance to passive movement.
    4. Observe for involuntary movements (eg, tremor, tics, fasciculations). Observe for resistance to passive movement; observe for decreased (flaccid) or increased (rigid/spastic) tone.

        Score: left and right

        Grade: **NORMAL, INCREASED or DECREASED**
B. Muscle Strength

1. Ask the subject to stand up from sitting without using hands *Grade:* ***NORMAL, IMPAIRED*** **and describe abnormality**
2. Test proximal limb strength by having the volunteer flex and extend the knees and elbows against your resistance.

   Test bilaterally and compare 1 side to the other.

   Score: left and right

   Grade: 5/5: normal; 4/5: movement against resistance impaired; 3/5: movement against gravity but not against resistance; 2/5: visible movement but not against gravity; 1/5: visible contraction; 0/5: no visible activity
3. Test distal limb strength by having the volunteer conduct dorsiflexion and plantar flexion of the volunteer's feet; finger abduction and handgrip strength against your resistance.

   Test bilaterally and compare 1 side to the other.

   Score: left and right

   Grade: 5/5: normal; 4/5: movement against resistance impaired; 3/5: movement against gravity but not against resistance; 2/5: visible movement but not against gravity; 1/5: visible contraction; 0/5: no visible activity

C. Pronator Drift
1. Ask the volunteer to hold both arms straight forward with, palms up and eyes closed for ~10-15 seconds as tolerated; watch for how well the arm position is maintained.
2. Instruct the volunteer to keep both arms still while you tap them briskly downward. The volunteer should normally be able to maintain extension and supination. Inability to maintain extension and supination (and drift into pronation) indicates an upper motor neuron deficit.

   Score: left and right

   Grade: **NORMAL or IMPAIRED and describe abnormality**

MODULE 4 - REFLEXES

A. Biceps

B. Knee

Note: *Other deep tendon reflexes may be tested at Investigator's discretion (eg elbow, wrist or Achilles tendon)*

Score: left and right

Grade: **NORMAL, INCREASED, DECREASED or ABSENT**

C. Babinski

Score: left and right

Grade: **NORMAL or ABNORMAL**

MODULE 5 - COORDINATION AND GAIT

A. Rapid, Rhythmic Alternating Movements

1. Testing each hand separately, ask the volunteer to tap the distal thumb with the tip of each finger, in sequence, as fast as possible.

   Score: left and right

   Grade: **NORMAL or IMPAIRED**

   Reminder: If the rapid alternate movements are disturbed, the subject will be asked to strike his hand on the thigh, raise the hand, turn it over and then strike the back of the hand down on the same place. (This test is impaired in cerebellar disease, extra pyramidal disease and upper MN weakness.)

B. Point-to-Point Movements

1. Ask the volunteer to touch your index finger and their nose alternately several times. Move your finger about as the volunteer performs this task.

   Score: left and right

   Grade: **NORMAL or IMPAIRED**

   Reminder: If the point-to-point testing is disturbed, the subject will be asked to place 1 heel on the opposite knee and then run it down the shin to the big toe. Repeat this for both sides. (Impaired tests indicate cerebellar disease.)

C. Romberg

1. Ask the volunteer to stand with both feet together and eyes closed for 20 to 30 seconds without support.
2. Be prepared to catch the volunteer if they are unstable.

   Grade: **NORMAL or IMPAIRED**

D. Gait

1. Ask the volunteer to walk across the room, turn and come back (assess posture, balance, swinging of arms and movement of the legs).

   Grade: **NORMAL or IMPAIRED and describe abnormality**

2. Ask the volunteer to walk heel-to-toe in a straight line (tandem gait).

   Grade: **NORMAL or IMPAIRED and describe abnormality**

MODULE 6 - SENSORY

A. Light touch sense: cotton wisp on skin of forearms and legs, bilaterally.

B. Pin prick: safety pin touched lightly to skin of forearms and legs, bilaterally.

C. Temperature: warm or cool object touched to skin of forearms and legs, bilaterally.

D. Vibration: tuning fork vibration detection in hands, feet bilaterally. E. Position sense: perception of thumb and toe movement, bilaterally.

F. Stereognosis: (identify common objects placed in hand, eg, coin, key).

Score: left and right

Grade: **NORMAL OR IMPAIRED and describe abnormality (for each A to F)**

12.3 Predefined Limits of Change Criteria

Predefined Limits of Change Criteria for Vital Signs, Weight, and Temperature

Measurement	Criteria
Systolic blood pressure	≥180 mm Hg and ≥20 mm Hg increase from baseline
	≤ 90 mm Hg and ≥ 20 mm Hg decrease from baseline
Diastolic blood pressure	≥105 mm Hg and ≥15 mm Hg increase from baseline
	≤ 50 mm Hg and ≥ 15 mm Hg decrease from baseline
Pulse	≥120 bpm and ≥15 bpm increase from baseline
	≤ 50 bpm and ≥ 15 bpm decrease from baseline
Orthostatic blood pressure	>20 mm Hg systolic sitting to standing after treatment (but not in baseline)
Weight	≥7 % increase from baseline
	≥7 % decrease from baseline
Temperature	≥101°F and ≥2°F increase from baseline (≥38.3°C and ≥1°C increase from baseline)

Predefined Limits of Change Criteria for ECGs

Measurement	Criteria
QTc Interval[†]	Prolongation compared to baseline ≥30 to ≤60 msec
	Prolongation compared to baseline >60 msec
	Value ≥500 msec[‡]
[2] Correction based on Bazett's formula. [3] The QTc Interval value must also represent a worsening compared to baseline to meet the definition.	

12.3 Mapping Between the 11 Categories of Suicidal Ideation and Behavior, the CSSRS, and CDR for Programming Standard Tables

Mapping Between the 11 Categories of Suicidal Ideation and Behavior and the C-SSRS

Category	C-SSRS Question (from eCRF)[†]
Suicidal ideation	
Passive	1. Wish to be dead
Active - nonspecific (no method, intent, or plan)	2. Non-specific active suicidal thoughts
Active - method, but no intent or plan	3. Active suicidal ideation with any methods (not plan) without intent to act
Active - intent, with or without a method, but no plan	4. Active suicidal ideation with some intent to act, without specific plan
Active - method, intent, and plan	5. Active suicidal ideation with specific plan and intent
Suicidal behavior	
Preparatory actions toward imminent suicidal behaviors	Preparatory acts or behavior
Aborted attempt	Aborted attempt
Interrupted attempt	Interrupted attempt
Suicide attempt	Actual attempt
Completed suicide	Completed suicide
Self-injurious behavior, no suicidal intent	Has subject engaged in non-suicidal self-injurious behavior?
[†]Data are "yes" or "no"	

13.0 SIGNATURES

13.2 Investigator

I agree to conduct this clinical trial in accordance with the design outlined in this protocol and to abide by all provisions of this protocol (including other manuals and documents referenced from this protocol). I agree to conduct the trial in accordance with generally accepted standards of Good Clinical Practice. I also agree to report all information or data in accordance with the protocol and, in particular, I agree to report any serious adverse events as defined in Section 7.0 – Assessing and Recording Adverse Events. I also agree to handle all clinical supplies and collect and handle all clinical specimens in accordance with the protocol. I understand that information that identifies me will be used and disclosed as described in the protocol, and that such information may be transferred to countries that do not have laws protecting such information. Since the information in this protocol and the referenced Investigator's Brochure is confidential, I understand that its disclosure to any third parties, other than those involved in approval, supervision, or conduct of the trial is prohibited. I will ensure that the necessary precautions are taken to protect such information from loss, inadvertent disclosure, or access by third parties.

TYPED NAME	
TITLE	
SIGNATURE	
DATE SIGNED	