Official Title: **PILOT STUDY TO EVALUATE THE EFFICACY AND SAFETY OF PLASMA EXCHANGE WITH ALBUTEIN® 5% IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS**

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CONFIDENTIAL

STATISTICAL ANALYSIS PLAN

Pilot Study to Evaluate the Efficacy and Safety of Plasma Exchange with Albutein® 5% in Patients with Amyotrophic Lateral Sclerosis

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Sponsor	Grifols
Product/Compound	Albutein® 5%
Phase of the study	IIa
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ABBREVIATIONS

AE	Adverse Event
ALS	Amyotrophic Lateral Sclerosis
ALS-CBS	Amyotrophic Lateral Sclerosis – Cognitive Behavioral Screen
ALSA-Q40	Amyotrophic Lateral Sclerosis Questionnaire 40
ALSFRS-R	Amyotrophic Lateral Sclerosis Functional Rating Scale – Revised
ALT	Alanine Transaminase
aPTT	Activated Partial Thromboplastin Time
AR	Adverse Reaction
AST	Aspartate Transaminase
ATC	Anatomical Therapeutic Chemical (Classification System)
BMAA	Beta-methylamino-L-alanine
CK	Creatinine Kinase
CSF	Cerebrospinal Fluid
DHMC	Dartmouth-Hitchcock Medical Center
EMA	European Medicines Agency
EMG	Electromyography
FVC	Forced Vital Capacity
HBsAg	Hepatitis B Surface Antigen
HCV	Hepatitis C Virus
HDL	High-Density Lipoprotein
HIV	Human Immunodeficiency Virus
INR	International Normalized Ratio
LDL	Low-Density Lipoprotein
MedDRA	Medical Dictionary for Regulatory Activities
NCS	Nerve Conduction Study
PE	Plasma Exchange
PP	Per Protocol
PT	Preferred Term
Q1	1st Quartile
Q3	3rd Quartile
SAP	Statistical Analysis Plan
SD	Standard Deviation
SOC	System Organ Class

TEAE	Treatment Emergent Adverse Event

1 INTRODUCTION

This Statistical Analysis Plan (SAP) is based on Clinical Study Protocol (CSP) Version 2.0, dated May 5th, 2016, and the SAP of the IG1309 study.

Amyotrophic Lateral Sclerosis (ALS) is an adult-onset paralyzing, rapidly progressive neurodegenerative disease that affects the upper and lower motor neurons. It is characterized by the loss of motor neurons in the cerebral cortex, brainstem and anterior horn of the spinal cord. ALS manifests itself with motor deficits, muscle atrophy, and difficult in breathing, and it is often accompanied by difficulty in swallowing.

ALS is the motor neuron disease with the worst prognosis, with an average survival after diagnosis that falls between 2 and 5 years, and 60% of patients dying within the 3 years (1,2). Death of ALS patients is usually produced by respiratory failure (3).

Two types of ALS are predominantly distinguished by their onset: limb-onset ALS which begins with motor impairments of the extremities, and bulbar-onset ALS with motor impairments at the level of the cranial nerves (4).

ALS continues to be a devastating neurodegenerative disease with an uncertain pathogenesis, for which no cure exists. There have not been any precise biomarkers identified for the disease, and diagnosis is established through clinical manifestations and neuronal damage evaluated through electromyography (EMG).

Current treatment for this disease is related to excitotoxicity control, which has limited results with regard to the prognosis (5). The first drug approved for its treatment is Riluzole, a glutamate release inhibitor, which lengthens life expectancy by 2 to 4 months in patients with moderate functional impairment (6-7). More recently Radicava® (edaravone) was approved by FDA in 2017 and is the only new FDA-approved treatment option for ALS in the last 20 years shown to slow disease progression (8). It is a a free radical scavenger, although the precise mechanism is unknown (9). Accordingly, the majority of interventions aimed at delaying the progression of the disease are at the symptomatic and/or palliative level. In this sense, care which helps preserve the quality of the subject's life is especially important and is mainly achieved via respiratory and nutritional support (10).

Among the clinical studies performed throughout the last 30 years, there were 3 pilot studies, which took place in the early 1980s, using plasmapheresis (a technique by which the plasma is separated from the formed blood components and discarded, and the components then returned to the subject) in a small number of ALS patients (11,12,13).

These studies made it possible to affirm that PE procedures are well tolerated by ALS patients. Nevertheless, they were not able to demonstrate efficacy due in part to methodological limitations that could be mitigated now, more than 30 years later, by using current knowledge of the disease and the experience acquired in ALS.

More specifically, some of the recruitment parameters used in the clinical design have been standardized (e.g., only including subjects having less than 18 months of disease progression and forced vital capacity (FVC) >70%) or the use of the ALSFRS-R functional scale as an efficacy variable.

The efficacy and safety of the plasmapheresis-based procedures have also improved, and their use has been expanded to numerous indications (14,15).

Instituto Grifols S.A. is currently studying the use of PE with Albutein in Alzheimer's disease (16). The working hypothesis stipulates that PE with albumin may change the metabolic profile

in ALS patients in both plasma and CSF; and in the case of CSF, this is thought to occur by altering the dynamic equilibrium between compartments. Thus, the potential benefits of PE may be due to the combination of the withdrawal of disease-inducing substances and to albumin's antioxidant effects and detoxifying functions via the transport and elimination of known and unknown harmful compounds. Furthermore, the study seeks to increase knowledge of potential new biomarkers for ALS.

However, disease progression has been found to be highly variable and does not always follow a linear descent (17,18). Therefore, the treatment will be considered efficacious when a subject's rate of decline is lower than the expected decline or disease progression assessed by ALSFRS-R.

For all these reasons, this pilot study was proposed with the objective of treating 10 ALS-diagnosed subjects, with PE using Albutein 5%.

Since ALS is a rapidly progressive disease, we plan to combine a first Intensive Treatment Phase followed by a Maintenance Treatment Phase in accordance with the progressive nature of the disease with the objective of obtaining disease stabilization or improvement.

2 STUDY OBJECTIVES

2.1 Primary Objective

To evaluate the disease progression using a Amyotrophic Lateral Sclerosis Functional Rating Scale – Revised (ALSFRS-R) and the forced vital capacity (FVC) of subjects affected by ALS treated with plasma exchange (PE) with Albutein 5%.

2.2 Secondary Objectives

To evaluate the effects of PE using Albutein 5% on:

- Cognitive dysfunction
- Beta-methylamino-L-alanine (BMAA) chronic toxicity
- Neurofilament levels
- Systemic inflammatory response

2.3 Safety Objectives

- Safety and tolerability of the procedure, including adverse events (AEs), clinical laboratory testing, physical examination, and vital signs.

3 OVERALL STUDY DESIGN

3.1 Overview of study design

This is a pilot, phase IIa, prospective, open-label and single-arm study, for a period of 12 months (six months of PE treatment with Albutein 5% and six months of follow-up after last PE).

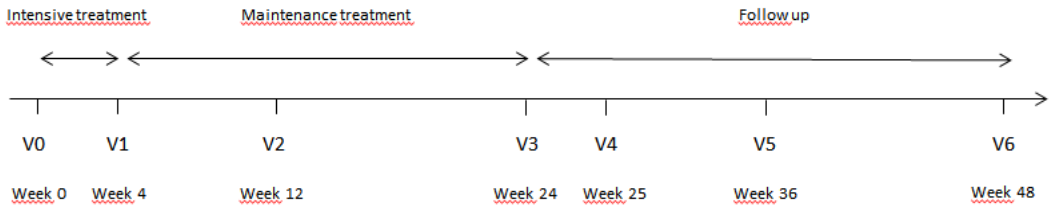
The six-month (24 weeks) treatment period consists in an Intensive Treatment Phase of 2 PEs per week over 3 weeks (6 PEs in total), followed by a Maintenance Treatment Phase of one

weekly PE for 21 weeks (21 PEs). In total, 27 PEs will be conducted during the overall treatment phase.

The following six visits are scheduled:

- Baseline Visit (V0), that will extend through a maximum of 3 weeks. Week 0 is defined as the week immediately prior to when the first PE is performed allowing to all other visits to be subsequently scheduled.
- Evaluation Visit (V1), will take place during week 4 once the Intensive Treatment Phase is completed, on the same day but before the PE#7 procedure.
- Evaluation Visit (V2), will take place at week 12 in the middle of the Maintenance Treatment Phase, before the PE#15 procedure in 2 consecutive days.
- Evaluation Visit (V3), will take place during week 24 for the purpose of finalizing the treatment period, on the same day but before the last scheduled PE#27 procedure.
- Follow-up Visit (V4), will take place 7 days after the last PE#27, on week 25, where the subject will come to the DHMC facilities.
- Follow-up Visit (V5), will be scheduled on week 36 (±7 days) corresponding to the middle point of the follow-up phase.
- Final Study Visit (V6), will take place during week 48 (±7 days) corresponding to the end of study.

The design can be visualized with the following chart:



More details are available in the Study Protocol concerning the examinations, tests, etc. that will be performed during each visit.

3.2 Study population

Subjects, males or females, who are older than 18 years of age and younger than 70, who have an ALS diagnosis, and who give their consent to participate after having been duly provided signed informed consent by the investigator may be included in this study.

Both untreated subjects and subjects on a stable dose of Riluzole for at least 28 days may be enrolled.

3.2.1 Inclusion criteria

1. Signed informed consent.

2. Subjects over 18 years of age and less than 70 years old.

3. Subjects with a possible, probable-lab supported, probable, or definite diagnosis of ALS, according to the revised El Escorial criteria.

4. Subjects having experienced their first ALS symptoms within 18 months prior to recruitment/consent.

5. FVC >70%.

6. Subjects must be medically suitable for study participation and willing to comply with all planned aspects of the protocol, including blood sampling, at the time of inclusion in the study.

3.2.2 Exclusion criteria

1. Subjects with pre-existing clinically significant lung disease not attributable to ALS.

2. Subjects diagnosed with other neurodegenerative diseases associated with other motor neuron dysfunction.

3. Participation in another investigational product study within one month prior to screening.

4. Females who are pregnant, breastfeeding, or, if of child-bearing potential, unwilling to practice a highly effective method of contraception (oral, injectable or implanted hormonal methods of contraception, placement of an intrauterine system, condom or occlusive cap with spermicidal foam/ gel/ film/ cream/ suppository, male sterilization, or true abstinence) throughout the study.

5. Difficult or problematic peripheral vein access and inability to implant a central catheter which would make continuous PE not feasible as per the visit protocol.

6. Contraindication to undergo PE or subject has abnormal coagulation parameters at the discretion of Outpatient Apheresis Unit team, including but not limited to:

    a) Thrombocytopenia (platelets <100,000/µL)

    b) Fibrinogen <1.5 g/L

    c) International Normalized Ratio (INR) >1.5

    d) Beta-blocker treatment and bradycardia <50 beats/min

    e) Treatment with angiotensin-converting enzyme inhibitors which may increase the risk of allergic reactions

7. History of anaphylaxis or severe systemic response to any plasma-derived albumin preparation, component of Albutein 5%, or other blood product(s).

8. Subjects unable to interrupt treatment with acetylsalicylic acid, other oral antiplatelet, or anticoagulant.

9. Renal dysfunction by elevated creatinine concentration >2 mg/dL.

10. Presence of heart disease that contraindicates PE treatment, including ischemic cardiopathy and congestive heart failure.

11. Presence of prior behavioral disorders requiring pharmacological intervention with less than 3 months of stable treatment.

12. Mentally challenged subject who cannot give independent informed consent.

13. Any condition that would complicate compliance with the study protocol (i.e., illness with the expectation of less than one year survival, abuse of drugs or alcohol, etc.).

3.3 Determination of sample size

There has been no formal calculation for the sample size since this is a pilot study. The number of subjects planned to be included in this study is 10 subjects completing the planned treatment. The sample size is considered adequate for the evaluation of the objectives of the study. In addition, there is a rising consensus in the ALS field that active control group comparisons should be avoided in early trials (19).

Lastly, the current EMA guidance document, *Guideline on clinical investigation of medicinal products for the treatment of amyotrophic lateral sclerosis (ALS) (EMA/CHMP/4015/2013)* states that the average functional decline is about 1 point per month in untreated subjects on the ALSFRS-R Functional Scale (20). Thus, it may be assumed that 10 subjects followed during 1 year of the clinical trial would be sufficient to evaluate a change of disease progression rate. In the event that a move towards stabilization is observed, the subsequent confirmatory studies will require a formal calculation for the sample size.

4 EFFICACY AND SAFETY VARIABLES

4.1 Primary efficacy variable

- Changes from baseline in the ALSFRS-R Functional Scale (5 post-baseline measurements: Weeks 4, 12, 25, 36, and 48),
- Changes from baseline in the FVC (5 post-baseline measurements: Weeks 4, 12, 25, 36, and 48).

4.2 Secondary efficacy variables

- Changes from baseline in cognitive function determined by the Amyotrophic Lateral Sclerosis – Cognitive Behavorial Screen (ALS-CBS) test (2 post-baseline measurements: Weeks 25 and 48),
- Changes from baseline in the motor evoked potential in thenar and hypothenar eminence and anterior tibialis muscle determined by electromyography (EMG) (5 post-baseline measurements: Weeks 4, 12, 25, 36, and 48),
- Evaluation of quality of life using the Amyotrophic Lateral Sclerosis Assessment Questionnaire 40 (ALSA-Q40) test (2 post-baseline measurements: Weeks 25 and 48),

- Changes from baseline in plasma cytokine panel and neurofilament analysis (8 post-baseline measurements: Weeks 4*, 12*, 24*, 36, and 48),
- Changes from baseline in Cerebrospinal Fluid (CSF) cytokine panel and neurofilament analysis (2 post-baseline measurements: Weeks 12 and 25)
- Changes from baseline in plasma BMAA levels (8 post-baseline measurements: Weeks 4*, 12*, 24*, 36, and 48),
- Changes from baseline in CSF BMAA levels (2 post-baseline measurements: Weeks 12 and 25),
- Changes from baseline in plasma immune population profile (3 post-baseline measurements: Weeks 4, 12, and 48).

* In those selected visits, there will be 2 measurements of biomarkers: before and after the PE procedure.

4.3 Safety variables

The following safety variables will be assessed:

- Percentage of PE sessions associated with at least one adverse reaction (AR) during or within 72 hours after the completion of the product infusion,
- Percentage of PE sessions temporarily associated with at least one AE, irrespective of causality, during or within 72 hours after the completion of the product infusion,
- Incidence of all AEs,
- Vital signs recorded at each assessment visit, before, during, and after each PE session, and as deemed necessary by the investigator,
- Pulse oximetry measurements recorded before, during, and after each PE session,
- Clinical laboratory testing (coagulation, blood count, biochemistry, and/or serology) at the specified visit.

5 DATA SETS TO BE ANALYSED

Subjects populations:

5.1 Enrolled Population

All recruited patients who provided written informed consent to participate.

5.2 Evaluable Population

Evaluable Population will include all subjects who receive at least one PE treatment with Albutein 5% and also have at least one baseline determination and a measurement of a primary efficacy variable at a subsequent visit: at least one measure of FVC and one measure of ALSFRS-R at baseline visit, and after the baseline visit.

5.3 Per-Protocol (PP) Population

All evaluable subjects who complete the treatment without major protocol deviations which could impact the primary efficacy assessment. The major protocol deviations will be determined in a data review meeting and will be documented in a data review report prior to the database lock.

5.4 Safety Population

All subjects who receive any amount of the study drug.

6 STATISTICAL AND ANALYTICAL PLANS

The tables and listings planned for the analysis are detailed in Appendix I.

6.1 Changes in the planned analyses

Any changes in the statistical analyses once the SAP has been finalised and after locking the database should be documented and justified on a file note and the clinical study report.

6.2 Blind review

Not applicable.

6.3 Hypotheses and statistical methods

6.3.1 Summary statistics

Data will be presented using summary statistics.

All categorical variables will be summarized in frequency and percentage.

The continuous variables will be reported by sample statistics: n (number of observations), number of missing data, mean, standard deviation (SD), minimum, first quartile (Q1), median, third quartile (Q3), and maximum. The 95% Confidence Interval for the mean will be added for the primary and secondary efficacy variables, as well as p-value for test comparisons when necessary (see sections 6.3.4 and 6.3.5 for details).

The number of digits behind the decimal point will be 1 in tables with percentages.

The summary statistics minimum and maximum will be presented to the same number of decimal places as that used to collect the data, while mean, median, Q1 and Q3 will be presented to one more decimal places, and standard deviation will be presented to two more decimal places.

All the analysis will be performed using SAS® Enterprise Guide system version 7.11.

6.3.2 Disposition of subjects

Subject disposition including the number of subjects enrolled in the study, number of subjects prematurely withdrawn from the study and the reasons of withdrawal will be presented.

The number of subjects included in the study, number of subjects included in evaluable, per protocol, and in safety populations, according to the definitions described in section 5, will be described, as well as the reasons from exclusion from each one of these populations.

Number of patients who fulfill or not the inclusion and the exclusion criteria will be presented, for each criteria described in section 3.2.

6.3.3 Demographic and other baseline characteristics

The following demographic and baseline data will be described at Baseline visit (V0):

- Age (see definition part 6.3.10), gender, race, ethnicity, height, weight
- ALS medical history: time since diagnosis, body part first affected by the disease, current status findings, and revised EI Escorial-Arlie Criteria
- Relevant medical and surgical history (presented in alphabetical order)
- Pregnancy test (performed yes/no, and result)
- Past assessment of primary endpoints: ALSFRS-R score and FVC score values before baseline visit, i.e. from the last 6 months prior to enrolment
- Peripheral venous access assessment

6.3.4 Primary efficacy analysis

The primary efficacy analyses will be carried out on the evaluable population, and will also be performed using the PP population.

For the primary efficacy variable, the ALSFRS-R Functional Scale, in addition to the overall score, values of the different individual questions, and functional subdomains will be summarized by visit.

For all these variables, and for the co-primary efficacy variable, FVC, changes from baseline will be summarized by visit.

Differences between baseline and subsequent visits for the ALSFRS-R overall and for the bulbar, fine/gross motor and respiratory subdomains will be analysed using the student t-test (normal distribution) or nonparametric Wilcoxon signed-rank test. Comparison between ALSFRS-R Overall change from baseline values obtained versus expected ALSFRS-R Overall change from baseline values according to EMA guideline, i.e. decrease of 1 point by month, will be analysed using the one-sample student t-test in the cases where the assumptions of normality are met, or nonparametric Wilcoxon signed-rank test if not.

Differences between baseline and subsequent visits for the Forced Vital Capacity (FVC), in liters and percentage will be analysed using the student t-test (normal distribution) or nonparametric Wilcoxon signed-rank test.

ALS Functional Rating Scale – Revised (ALSFRS-R):

This test is a validated rating instrument for progression of disability in ALS that provides an estimate of the degree of the subject's functional impairment through a short questionnaire. The ALSFRS-R includes 12 questions to assess the level of self-sufficiency of subjects in various functional domains. Aspects of nourishment, personal care, personal autonomy, and communication are also evaluated. Each task is graded on a five point scale from 0 (not able to do) to 4 (normal ability). Individual scores are totaled to produce a final result of between 0 (worst) and 48 (best).

ALSFRS-R will be classified as following, between 3 subdomains:

- Bulbar function (questions #1 to #3),
- Fine and gross motor function (questions #4 to #9),
- Respiratory function (questions #10 to #12).

Note: item #5 (cutting food) is divided into 2 sub-items: #5a (without gastrotomy) and #5b (with gastrotomy). Only one of them can be filled, and the other one should be missing.

In case of any item is missing, the total score and the corresponding subdomain score will be considered missing.

Pulmonary Function: Forced Vital Capacity (FVC):

The FVC measures the volume of air that a person can forcibly exhale through a spirometer after a full inspiration. Following the the recommendations of the American Thoracic Society and European Respiratory Society this should be measured in a seated position, which facilitates subjects to be able perform multiple measurements if necessary. The result obtained is expressed in a percentage over the expected result in the general population.

6.3.5 Secondary efficacy analyses

Changes from baseline in EMG profile (sensory NCS and motor NCS), ALS-CBS, ALSA-Q40, and different biomarkers such as cytokine panel (plasma/CSF), neurofilament (plasma/CSF), BMAA (plasma/CSF), and immune population profile (plasma) will be summarized by visit.

In addition, changes in the EMG profile, ALS-CBS, ALSA-Q40, plasma cytokine panel, plasma neurofilament levels, and plasma BMAA levels before and after selected PE (PE#7, PE#15, and PE#27) will be summarized and analyzed using the non-parametric Wilcoxon signed-rank test for paired data.

ALS Cognitive Behavorial Screen (ALS-CBS):

This test is designed as a screening tool to help identify subjects at risk for frontotemporal cognitive impairment and/or behavioral disorders. As such, this tool should not be used to establish a diagnosis, and a clinical neurological assessment will be required for diagnosis of ALS on all subjects prior to the test.

The test is composed by two sections. The first section is a 4 subdomain cognitive screening consisting of: Attention (complex orders, mental sums, language, and eye movement); Concentration (inversion of numeric series); Follow-up and monitoring (reverse sequences, alphabet, number and letter sequencing); and Initiation and Recovery (nomination). The second section consists of a questionnaire with 15 items of behavioral changes assessed by the caretaker.

The result of the cognitive section is a score of 0 to 20, based on the accuracy of the responses and the errors committed. The result of the behavioral section is the sum of the items on a Likert Scale.
A cognitive screening score will be computed, as the sum of the 4 items of the cognitive section (test 2): attention, concentration, tracking and monitoring, initial and retrieval.

In case of any item is missing, the cognitive score will be considered missing.

ALS Assessment Questionnaire 40 (ALSA-40):

This specific ALS quality of life questionnaire provides insight on situations of great importance for ALS subjects in areas such as mobility, fear of falling when walking, difficulty eating and cutting, participation in meetings, feelings of isolation, embarrassing social situations, feelings of fear and hopelessness in the future, and problems associated with motor neuron disease.

The questionnaire consists of 40 items grouped into 5 representative dimensions associated with quality of life:

- Physical mobility (questions #1 to #10),
- Activities of daily living (questions #11 to #20),
- Food and drink (questions #21 to #23),
- Communication (questions #24 to #30),
- Emotional function (questions #31 to #40).

Each question is scored from 0 to 4 according to a gradation of symptom onset frequency (never, rarely, sometimes, often, and always). From raw scores, an index from 0 to 100 is obtained for each dimension, which make comparisons with the other dimensions possible as well as a straightforward interpretation of the results (0 = better state of health as measured by the questionnaire;100 = poorer state of health). The first 4 scales refer to deficits and subsequent disabilities as a result of the disease. The fifth scale reflects how the subject is facing his/her physical deterioration emotionally.

Data for ALSA-40 will be presented using raw scores (original values, i.e. physical mobility ranging from 0 to 40, food and drink ranging from 0 to 12), and using transformed scores (all the 5 dimensions scores ranging from 0 to 100).

Electromyography (EMG)

Surface EMG will be performed to record motor evoked potential in the distal muscles of the upper limbs (thenar and hypothenar eminence) and dorsiflexor muscles of the lower limbs (anterior tibialis) after electrical stimulation on the median, ulnar, and external popliteal sciatic nerve. Sensory nerve conduction studies (NCS), Motor NCS will be listed.

CSF and plasma samples

Values and change from baseline values will be summarized by visit. The following parameters will be evaluated:

- CSF standard analysis (see in “Other safety assessments”, section 6.3.9)
- Plasma cytokine panel
- CSF cytokine panel
- Plasma neurofilament analysis
- CSF neurofilament analysis

- Plasma BMAA levels
- CSF BMAA levels
- Plasma immune population profile

6.3.6 Exposure to treatment

Plasma exchange

The following information will be described at each PE assessment: duration of PE, route used, batch number of albumin, subject weight, product amount, exchange volume, exchange speed.

The number of PE received by visit and during the study will be summarized.

6.3.7 Concomitant medication

All medications will be coded using Anatomical Therapeutic Chemical (ATC) classification codes via the World Health Organization Drug classification Dictionary (WHO-DD), latest version. All medications will be summarized and sorted alphabetically by medication class (i.e., ATC level 2) and medication sub-class (i.e., ATC level 4). If the ATC level 4 term is missing, the ATC level 3 term will be used in the medication summary table and data listing.

Prior medications and concomitant medications will be summarized separately. Prior medications are defined as any medication ended prior to the start of study treatment (i.e., start of PE#1). Concomitant medications are defined as any medication started on or after the start of study treatment or any medication taken prior to the start of study treatment and continued after the start of study treatment during the study.

6.3.8 Adverse events

The Safety Analysis will be based on the safety population.

For summary purposes, adverse events (AEs) will be classified as treatment emergent AEs (TEAEs) or non-treatment emergent AEs (non-TEAEs) depending on the comparison of AE onset date/time with the start of study treatment treatment (i.e., start of PE#1). A TEAE will be defined as an AE which occurred on or after the start of study treatment. Non-TEAEs and TEAEs will be summarized separately.

The number of subjects with any TEAE and the total number of TEAEs will be presented in a summary table, and the characteristics of these adverse events will be described: number of subjects with, and total number of: serious TEAEs, TEAEs leading to death, severe TEAEs, TEAEs related to study drug, TEAEs related to study procedure, TEAEs leading to discontinuation from the study.

In a similar table, the number of subjects with, and the total number of: non-TEAEs, serious non-TEAEs, severe non-TEAEs will be presented.

The proportion of PE sessions temporarily associated with at least one AR (defined as occurring during or within 72 hours after the completion of the product infusion) will be summarized.

The proportion of PE sessions temporarily associated with at least one TEAE, irrespective of causality, during or within 72 hours after the completion of the product infusion, will also be summarized.

The number of subjects with any TEAE will be tabulated and summarized. AEs will be coded in accordance with the Medical Dictionary for Regulatory Activities (MedDRA) AE classification (version 17.0 or higher), and will be tabulated by system organ class (SOC) and preferred term (PT), presented in alphabetic order. This will be done overall, and by severity, causality, and seriousness levels.

Severity (or intensity) of AE:

AEs are classified according to their severity gradation as Mild, Moderate, and Severe.

Causality (relationship) of AE:

AEs will be considered related to the study drug when causality is “definitive”, “probable”, “possible” or “doubtful/unlikely” and such an event will be defined as a suspected AR. A suspected AR with a causal relationship of “definite” will be defined as an AR. On the other hand, when causality is “unrelated”, AEs will be considered as not related to the study drug.

Same rules will apply to define AEs related to study procedure.

In the table presented by causality, 4 classes of causality will be defined:

- related to study drug only
- related to study procedure only
- related to both study drug and study procedure
- not related

Seriousness of AE:

AEs will be distinguished between serious and non-serious AEs, according to the view of either the investigator or Sponsor, depending on its outcome.

Each subject is counted once within each unique SOC and PT level. For example, if a subject has two headaches, the subject is counted only once under the PT “Headache” (worst case in terms of severity, causality, and seriousness will be chosen). Likewise, each subject is counted only once at each SOC level although they may have several different PT events within the same SOC.

6.3.9 Other safety assessments

Vital signs

Vital signs (blood pressure, body temperature, heart rate, and respiratory rate) will be recorded at each assessment visit, 10-15 minutes before, during (30 min after PE procedures starts), and 15-30 minutes after each session of PE, and as deemed necessary by the investigator.

Absolute values and change from baseline of vital signs through the study will be summarized by parameter and then by visit/week; and for the vital signs from PE sessions, the pre-PE

values will be used to compare with baseline. In addition, absolute values from the 3 vital sign assessments and change from pre-PE to during-PE and after-PE will be summarized for all PE sessions.

Pulse Oximetry

Blood oxygen will be recorded using a pulse oximeter 10-15 minutes before, during (30 min after PE procedures starts), and 15-30 minutes after each session of PE.

Absolute values and change from baseline of pulse oximetry through the study will be summarized by parameter and then by visit/week; and for the pulse oximetry values from PE sessions, the pre-PE values will be used for the summary and comparison with baseline. In addition, absolute values from the 3 pulse oximetry assessments and change from pre-PE to during-PE and after-PE will be summarized for all PE sessions.

Physical Examination

Physical examination (if normal, abnormal, clinically significant) will be described and listed on an individual basis: skin, eyes/ nose/ throat/ ear, head/ neck, thorax, abdomen, extremities and joints, respiratory, cardiac, renal and urinary, neurological, other. In the summary table, entries for 'Other' body systems will be grouped together; a subject with 2 or more 'Other' entries will be counted only once.

Neurological Examination

Neurological examination will be described: cranial nerves (oculomotor, facial), motor function (muscles), reflexes (in both arms and both legs), and sensation assessment.

Laboratory Safety Assessments

Clinical laboratory parameters and changes from baseline values will be summarized by visit. This analysis will include parameters of:

- Complete Blood Count: hematocrit, hemoglobin, erythrocytes, platelets, leucocytes, neutrophils, lymphocytes, monocytes, eosinophils, basophils
- Coagulation: prothrombin time, aPTT
- Fibrinogen
- Biochemistry and Lipid Profile: alanine transaminase (ALT), aspartate transaminase (AST), creatine kinase (CK), creatinine, ferritin, glucose, protein (pre-albumin, albumin, total protein in serum), and total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and triglycerides
- Serum electrolytes: calcium, phosphate, sodium, potassium, chloride, bicarbonate
- Serology: HIV 1 & 2, HCV antibodies, and HBsAg antigen

Shift tables will show the number of patients who changed from xxxxx.

CSF standard analysis

Values and change from baseline values will be summarized by visit. The following parameters will be evaluated: cell count (leucocytes, neutrophils, lymphocytes, monocytes), glucose, protein, IgG (mass), and albumin levels.

6.3.10 Definitions

1. **Age**

Age of the subject will be defined in years, as:

$$\text{Age} = \frac{(\text{date of informed consent signed - date of birth} + 1)}{365.25}$$

2. **Time since diagnosis**

Time since diagnosis will be reported in months, as the period of time between diagnosis date to the date of informed consent signed.

$$\text{Time since diagnosis} = \frac{(\text{date of informed consent signed - diagnosis date} + 1)}{30.4375}$$

3. **Baseline and Change from baseline**

Baseline will be defined as the measurement taken at the Baseline visit or the last measurement taken prior to the start of the study treatment (i.e. PE#1) if the parameter is not measured at the Baseline visit or there are multiple observations for the parameter at the Baseline visit.

Change from baseline values will be reported as the difference between: value at the visit minus value at baseline visit.

6.4 Level of significance, multiple comparisons and multiplicity

If applicable, all statistical tests will be 2-sided and will be performed using a 5% significance level.

No adjustment for multiple comparisons or corrections for multiplicity are planned.

6.5 Adjustment for covariates

Not applicable.

6.6 Handling of dropouts and missing data

No imputation algorithms will be adopted to replace missing values.

In all listings, missing or incomplete dates should be left as they have been recorded. However, for calculation / sorting / assignation based on dates, the following methods will be used:

1) The most conservative approach will be systematically considered (i.e. if the onset date of an AE/concomitant medication is missing/incomplete, it is assumed to have occurred

during the study treatment phase (i.e. a TEAE for AEs) except if the partial onset date or other data [stop date,…] indicates differently).

2) A missing/incomplete date of medical history or disease diagnosis will be assumed to have occurred before any study treatment.

(3) In the event that any date is incomplete after Data Management processing and this date needs to be used for calculation (eg. time since primary diagnosis), the following will be assigned for the incomplete date:

- if no field is available: no imputation will be performed.
- if only the year is available: Day “01” and month of “July” will be imputed.
- if the month and year are available: Day “15” will be imputed.

6.7 Multicentre studies

Not applicable.

6.8 Examination of subgroups

For both co-primary efficacy variables (ALSFRS-R and FVC), subgroup analysis will be performed, based on Riluzole use (yes or no) and site of onset (limb-onset or bulbar onset).

6.9 Interim analysis

No interim analysis will be performed.

6.10 Data monitoring

Not applicable.

7 REFERENCES

[1] Mitchell JD & Borasio GD. Amyotrophic lateral sclerosis. Lancet. 2007; 369:2031-41.

[2] Talbot K. Motor neuron disease: the bare essentials. Pract Neurol. 2009; 9:303-09.

[3] Kiernan MC, Vucic S, Cheah BC, Turner MR, Eisen A, Hardiman O, Burrell JR, Zoing MC. Amyotrophic lateral sclerosis. Lancet. 2011; 377: 942–55.

[4] Andrews J. Amyotrophic lateral sclerosis: clinical management and research update. Curr Neurol Neurosci Rep. 2009; 9 (1): 59-68.

[5] Brooks BR. Managing amyotrophic lateral sclerosis: slowing disease progression and improving patient quality of life. Ann Neurol. 2009; 65 (Suppl. 1): S17-23.

[6] Miller RG, Mitchell JD, Lyon M, Moore DH. Riluzole for amyotrophic lateral sclerosis (ALS)/,motor neuron disease (MND). Cochrane Database Syst Rev. 2007; 1:CD001447.

[7] Bensimon G, Lacomblez L, Delumeau JC, Bejuit R, Truffinet P, Meininger V. A study of riluzole in the treatment of advanced stage or elderly patients with amyotrophic lateral sclerosis. J Neurol. 2002; 259:609-15.

[8] Package insert 2018 Edaravone.

[9] Oskarsson B, Gendron TF, Staff NP. Amyotrophic Lateral Sclerosis: An Update for 2018. Mayo Clin Proc. 2018;93(11):1617-1628. doi: 10.1016/j.mayocp.2018.04.007.

[10] Hardiman O. Symptomatic treatment of respiratory and nutritional failure in amyotrophic lateral sclerosis. J Neurol. 2000; 257: 245-51.

[11] Monstad I, Dale I, Petlund CF, Sjaastad O. Plasma exchange in motor neuron disease. A controlled study. J Neurol. 1979; 221(1):59-66.

[12] Silani V, Scarlato G, Valli G, Marconi M. Plasma exchange ineffective in amyotrophic lateral sclerosis. Arch Neurol. 1980; 37(8):511-3.

[13] Kelemen J, Hedlund W, Orlin JB, Berkman EM, Munsat TL. Plasmapheresis with immunosupression in amyotrophic lateral sclerosis. Arch Neurol. 1983;4 0(12):752-3.

[14] Szczepiorkowski ZM, Winters JL, Bandarenko N, Kim HC, Linenberger ML, Marques MB, Sarode R, Schwartz J, Weinstein R, Shaz BH. Guidelines on the use of therapeutic apheresis in clinical practice – Evidence-based approach from the apheresis applications committee of the American Society for Apheresis. J Clin Apheresis. 2010; 25:83-177.

[15] Anaya F, Aféresis Terapéutica. Memoria año 2012. Hospital general universitario Gregorio Marañón.http://www.senefro.org/modules.php?name=grupos&d_op=viewgroup&idgroup=10949 (visited 2 april 2013).

[16] Boada M, Ortiz P, Anaya F, Hernández I, Muñoz J, Núnez L, Olazarán J, Roca I, Cuberas G, Tárraga L, Buendia M, Pla RP, Ferrer I, Páez A. Amyloid-targeted therapeutics in alzheimer's disease: use of human albúmina in plasma Exchange as a novel approach for Aâ mobilization. Drug News Perspect. 2009; 22(6):325-39.

[17] Gordon PH, Cheng B, Salachas F, Pradat PF, Bruneteau G, Corcia P, Lacomblez L, Meininger V. Progression in ALS is not linear but is curvilinear (2010). J Neurol; 257-1713-7.

[18] Gomeni R & Fava M. Amyotrophic lateral sclerosis disease progression model. 2014 Amyotroph Lateral Scler Frontotemporal Degener;15(1-2):119-29.Bowser R, Turner MR, Shefner J. Biomarkers in amyotrophic lateal sclerosis: opportunities and limitations. Nat Rev Neurol. 2011;7(11):631-8.

[19] Gladman M, Cudkowicz M, Zinman L. Enhancing clinical trials in neurodegenerative disorders: lessons from amyotrophic lateral sclerosis. Curr Opin Neurol. 2012 Dec;25(6):735-42.

[20] Guideline on clinical investigation of medicinal products for the threatment of amyotrophic lateral sclerosis (ALS) EMA/CHMP/40105/2013. July 2013.

8 APPENDIX 1 – DATA PRESENTATION PLAN

8.1 Tables to be produced for the Clinical Study Report (Section 14 according to ICH E3)

Demographic and baseline characteristics

Table 14.1.1	Inclusion and exclusion criteria – Enrolled Population
Table 14.1.2	Disposition of subjects – Enrolled Population
Table 14.1.3	Reason for exclusion from population – Enrolled Population

Table 14.1.4 Demographics data – Safety Population

Table 14.1.5 ALS Medical history – Evaluable Population

Table 14.1.6 Relevant medical/surgical history – Safety Population

Table 14.1.7 Pregnancy test – Safety Population

Table 14.1.8 Peripheral venous access assessment – Evaluable Population

Primary Efficacy analysis

Table 14.2.1.1 ALS Functional Rating Scale by visit – Evaluable population.

Table 14.2.1.2 ALS Functional Rating Scale by visit – Per-Protocol population.

Table 14.2.1.3 ALS Functional Rating Scale by visit and Riluzole use – Evaluable population.

Table 14.2.1.4 ALS Functional Rating Scale by visit and Riluzole use – Per-Protocol population.

Table 14.2.1.5 ALS Functional Rating Scale by visit and site of onset – Evaluable population.

Table 14.2.1.6 ALS Functional Rating Scale by visit and site of onset – Per-Protocol population.

Table 14.2.2.1 Pulmonary function by visit – Evaluable population.

Table 14.2.2.2 Pulmonary function by visit – Per-Protocol population.

Table 14.2.2.3 Pulmonary function by visit and Riluzole use – Evaluable population.

Table 14.2.2.4 Pulmonary function by visit and Riluzole use – Per-Protocol population.

Table 14.2.2.5 Pulmonary function by visit and site of onset – Evaluable population.

Table 14.2.2.6 Pulmonary function by visit and site of onset – Per-Protocol population.

Secondary Efficacy analysis

Table 14.2.3.1 Electromyography: sensory NCS – Evaluable Population

Table 14.2.3.2 Electromyography: motor NCS – Evaluable Population

Table 14.2.4.1 ALS Assessment Questionnaire 40 (ALSA Q40): raw scores – Evaluable Population

Table 14.2.4.2 ALS Assessment Questionnaire 40 (ALSA Q40): transformed scores – Evaluable Population

Table 14.2.5 ALS Cognitive Behavioral Screen (ALS-CBS) – Evaluable Population

Table 14.2.6 CSF biomarkers (cytokine panel, neurofilament analysis, BMAA levels) – Evaluable Population

Table 14.2.7 Changes in the plasma biomarkers before and after selected PE – Evaluable Population

Adverse events

Table 14.3.1.1 Summary of non-TEAEs – Safety Population

Table 14.3.1.2 Summary of TEAEs – Safety Population

Table 14.3.1.3 PE sessions temporarily associated with AR, occurring during or within 72 hours after the completion of the product infusion – Safety Population

Table 14.3.1.4 PE sessions associated with TEAE, occurring during or within 72 hours after the completion of the product infusion – Safety Population

Table 14.3.1.5 TEAEs: MedDRA coding by SOC and PT – Safety Population

Table 14.3.1.6 TEAEs: MedDRA coding by severity, SOC and PT – Safety Population

Table 14.3.1.7 TEAEs: MedDRA coding by causality, SOC and PT – Safety Population

Table 14.3.1.8 TEAEs: MedDRA coding by seriousness, SOC and PT – Safety Population

List of deaths and other serious adverse events

Table 14.3.2.1 Adverse events leading to death – Enrolled Population

Table 14.3.2.2 Adverse events leading to discontinuation from the study – Enrolled Population

Table 14.3.2.3 Serious adverse events – Enrolled Population

Laboratory data

Table 14.3.3.1 Complete Blood Count: absolute values and change from baseline by visit – Safety Population

Table 14.3.3.2 Complete Blood Count: summary of shift of values by visit – Safety Population

Table 14.3.3.3 Biochemistry and Lipid Profile: absolute values and change from baseline by visit – Safety Population

Table 14.3.3.4 Biochemistry and Lipid Profile: summary of shift of valuesby visit – Safety Population

Table 14.3.3.5 Serum Electrolytes: absolute values and change from baseline by visit – Safety Population

Table 14.3.3.6 Serum Electrolytes: summary of shift of values by visit – Safety Population

Table 14.3.3.7 Coagulation: absolute values and change from baseline by visit – Safety Population

Table 14.3.3.8 Coagulation: summary of shift of values by visit – Safety Population

Table 14.3.3.9 Fibrinogen: absolute values and change from baseline by visit – Safety Population

Table 14.3.3.10 Fibrinogen: summary of shift of values by visit – Safety Population

Table 14.3.3.11 Serology results by visit – Safety Population

Concomitant therapy

Table 14.3.4.1 Prior medication – Safety Population

Table 14.3.4.2 Concomitant medication – Safety Population

Other safety data

Table 14.3.5.1 Vital signs during evaluation visits: absolute and change from baseline values by visit – Safety Population

Table 14.3.5.2 Vital signs during PE: change from pre-PE assessments – Safety Population

Table 14.3.5.3 Pulse oximetry during PE: change from pre-PE assessment – Safety Population

Table 14.3.5.4 Plasma exchanges by visit – Safety Population

Table 14.3.5.5 Number of PEs received during the study – Safety Population

Table 14.3.5.6 CSF standard analysis: absolute values and change from baseline by visit – Safety Population

Table 14.3.5.7 CSF standard analysis: abnormalities by visit – Safety Population

Other relevant data

Table 14.3.6 Physical examination by visit – Safety Population

Table 14.3.7 Neurological examination by visit – Evaluable Population

8.2 Graphs (Section 14.2-14.3 in ICH E3)

Figure 14.4.1 ALS Functional Rating Scale by visit – Evaluable population.

Figure 14.4.2 ALS Functional Rating Scale by visit – Per-Protocol population.

Figure 14.4.3 Pulmonary function by visit – Per-Protocol population.

Figure 14.4.4 Pulmonary function by visit – Evaluable population.

Figure 14.4.5 Past assessment of ALSFRS-R scores – Evaluable Population

Figure 14.4.6 Past assessment of FVC scores – Evaluable Population

8.3 Listings of individual subject data and other information to be produced for the Clinical Study Report (Sections 16.1 and 16.2 according to ICH E3)

16.2.1. Discontinued subjects.

16.2.1.1 Study termination

16.2.1.2 Treatment termination

16.2.1.3 Subject discontinuation

16.2.1.4 Visit dates

16.2.2. Protocol deviations.

16.2.2	Protocol deviations

16.2.3. Subjects excluded from the analysis.

16.2.3	Subjects excluded from any analysis population

16.2.4. Demographic data.

16.2.4.1	Demographic data
16.2.4.2	ALS Medical history
16.2.4.3	Relevant medical/surgical history
16.2.4.4	Pregnancy test
16.2.4.5	Past assessment of primary endpoints
16.2.4.6	Peripheral venous access assessment
16.2.4.7.1	Inclusion and exclusion criteria
16.2.4.7.2	Eligibility

16.2.5. Individual efficacy response data.

16.2.5.1	ALS Functional Rating Scale
16.2.5.2	Pulmonary function data
16.2.5.3	Electromyography: sensory NCS
16.2.5.4	Electromyography: motor NCS
16.2.5.5	ALSA Q-40 data
16.2.5.6	ALS Cognitive Behavioral Screen (ALS-CBS) data
16.2.5.7	Plasma biomarkers collection
16.2.5.8	CSF biomarkers collection

16.2.6. Adverse events.

16.2.6.1	Adverse events

16.2.7. Individual laboratory measurements.

16.2.7.1	Complete Blood Count measurements data
16.2.7.2	Biochemistry measurements data
16.2.7.3	Serum Electrolytes measurements data
16.2.7.4	Coagulation measurements data
16.2.7.5	Fibrinogen measurements data

16.2.7.6 Serology measurements data

16.2.8. Vital signs and Pulse Oximetry

16.2.8.1 Vital signs

16.2.8.2 Pulse Oximetry

16.2.9. Concomitant medications.

16.2.9.1 Prior medications

16.2.9.2 Concomitant medications

16.2.10. Other relevant data.

16.2.10.1 Plasma exchange

16.2.10.2 Physical examination

16.2.10.3 Neurological examination

16.2.10.4 CSF standard analysis data