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

NCT Number: NCT02872142

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CLINICAL STUDY PROTOCOL

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

Protocol Number/ Protocol Version Number/Date:	GBI1501 / Version 2.0 (including Protocol Amendment 1) / 05 May 2016
Name of the Investigational Product:	Albutein® 5%
Name and address of manufacturer:	GRIFOLS BIOLOGICALS Inc. 5555 Valley Boulevard Los Angeles, CA 90032
Sponsor Identification:	GRIFOLS BIOLOGICALS Inc. 5555 Valley Boulevard Los Angeles, CA 90032
Name and address of the Principal Investigators:	[redacted], MD. Dartmouth-Hitchcock Medical Center One Medical Center Drive Lebanon, New Hampshire 03756 [redacted], MD, PhD. Dartmouth-Hitchcock Medical Center One Medical Center Drive Lebanon, New Hampshire 03756

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

SIGNATURE SHEET

Sponsor

[redacted] 05 May 2016

[redacted] *PhD*

Date

Grif[redacted] Group

09 May 2016

[redacted] *MD*

Date

Grifols Bioscience Industrial Group

Summary of Changes for Protocol Amendment 1

Protocol Version	Date of Finalization
2.0 Including Amendment 1	05 May 2016
1.0 Original	24 Jul 2015

Version 2.0, 05 May 2016

Pilot study to evaluate the efficacy and safety of plasma exchange with albutein® 5% in patients with amyotrophic lateral sclerosis

The protocol for Study GBI1501 (Version 1.0 dated 24 Jul 2015) has been amended and reissued as Protocol Version 2.0, dated 05 May 2016

AMENDMENT 1 – SUMMARY OF CHANGES

(Note: Administrative changes including minor administrative corrections are not included in this Summary of Changes.)

Sections	Change From: (Version 1.0 dated 24 Jul 2015) (Strikethrough is added to highlight deleted text)	Change To: (Version 2.0, dated 05 May 2016) (Underline is added to highlight new text)	Rationale:
Protocol Synopsis Exclusion Criteria Section 4.1.2	5. Difficult or problematic peripheral vein access ~~restricting the~~ ability to implant a 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 the ~~a~~pheresis team ~~(DHMC)~~, including but not limited to: e) Treatment with angiotensin-converting enzyme inhibitors which may increase the risk of allergic reactions~~, unless a preventive change in hypotensive treatment occurs prior to enrollment~~	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 the Outpatient Apheresis Unit team, including but not limited to: e) Treatment with angiotensin-converting enzyme inhibitors which may increase the risk of allergic reactions	#5: Revised to provide clarification in response to the FDA information request and recommendations. #6: The specific location where PE procedures will take place was added in place of the general statement of DHMC (Dartmouth-Hitchcock Medical Center). e) Revised to provide clarification in response to the FDA information request and recommendations.
Protocol Synopsis: Treatment modes of administration	The PE procedure will be preferably performed via peripheral venous access for the administration of Albutein 5%.	The PE procedure will be preferably performed via peripheral venous access for the administration of Albutein 5%, although subjects will be allowed to switch to a central catheter during the treatment phase if repetitive problems to maintain exchange rate are encountered (see section 5.2 for details).	Clarification was added to explain the preference of starting all subjects with peripheral venous access, but allowing the switch to central catheter.
Protocol Synopsis: Study Variables Section 6.1.3	No previous text	- Pulse oximetry measurements determined before, during, and after each PE session	Pulse oximetry measurements added according to FDA information request and recommendations.

Sections	Change From: (Version 1.0 dated 24 Jul 2015) (Strikethrough is added to highlight deleted text)	Change To: (Version 2.0, dated 05 May 2016) (Underline is added to highlight new text)	Rationale:
Section 1.2	More recently, in 2008 a clinical case was reported involving a patient with elevated anti-phospholipid antibody titers who was diagnosed as definite ALS based on the El Escorial ~~Arlie scale~~.(18)	More recently, in 2008 a clinical case was reported involving a patient with elevated anti-phospholipid antibody titers who was diagnosed as definite ALS based on the revised El Escorial criteria.(18)	Changed for consistency across the document.
Section 4.2	According to the El Escorial ~~Arlie~~ criteria, the ALS diagnosis consists of: No previous text	According to the revised El Escorial criteria, the ALS diagnosis consists of: Therefore several categories of ALS diagnosis can be described: – **Clinically definite ALS:** clinical evidence alone of upper and lower motor neuron signs in three regions. – **Clinically probable ALS:** clinical evidence alone of upper and lower motor neuron signs in at least two regions with some upper motor neuron signs rostral to (above) the lower motor neuron signs. – **Clinically probable–laboratory-supported ALS**: clinical signs of upper and lower motor neuron dysfunction are in only one region, or upper motor neuron signs alone in one region with lower motor neuron signs defined by electromyography criteria in at least two limbs, together with proper application of neuroimaging and clinical laboratory protocols to exclude other causes. – **Possible ALS:** clinical signs of upper and lower motor neuron dysfunction in only one region, or upper motor neuron signs alone in two or more regions; or lower motor neuron signs rostral to upper motor neuron signs and the diagnosis of clinically probable-laboratory-supported ALS	Changed for consistency across the document. Added according to FDA information request and recommendations.

Sections	Change From: (Version 1.0 dated 24 Jul 2015) (Strikethrough is added to highlight deleted text)	Change To: (Version 2.0, dated 05 May 2016) (Underline is added to highlight new text)	Rationale:
		cannot be proven.	
Section 4.4.	4.4. Withdrawal criteria 9. Subjects who develop an infection with hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV), or human Immunodeficiency virus (HIV) during the study. Though subjects will not be routinely tested for these pathogens during the trial, they will be under a physician's supervision throughout the study. A subject should be withdrawn if, in the opinion of his/her physician, he/she develops any of these viral infections. No previous text	4.4. Withdrawal criteria and stopping rules 9. Subjects who develop an infection with hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV), or human Immunodeficiency virus (HIV 1 & 2) during the study. Though subjects will not be routinely tested for these pathogens during the trial, they will be under a physician's supervision throughout the study. A subject should be withdrawn if, in the opinion of his/her physician, he/she develops any of these viral infections. Under the following circumstances the study team will recommend to halt the trial temporarily (until modifications to the protocol are completed) or wholly (abandon all study activities): – Temporarily halt the trial if more than 30% of subjects undergoing plasma exchange procedures have an SAE as defined as related to the Investigational Product (remember that if causal relationship of the SAE is labeled as "definite", "probable", "possible" or "doubtful/unlikely", the event will be defined as a suspected adverse drug reaction) and not attributable to the therapeutic procedure or technique of application, as judged by the principal investigator and Sponsor, requiring that both parties agree. – Completely stop the trial if more than 50% of subjects undergoing plasma exchange procedures have an SAE as defined as related to the	Added additional circumstances for study stopping rules according to FDA information request and recommendations.

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		Investigational Product (remember that if causal relationship of the SAE is labeled as "definite", "probable", "possible" or "doubtful/unlikely", the event will be defined as a suspected adverse drug reaction) and not attributable to the therapeutic procedure or technique of application, as judged by the principal investigator and Sponsor, requiring that both parties agree.	
Section 5.2	This study will evaluate the effects of PE with Albutein 5% in treating ALS. ~~In an attempt to prevent the development of coagulopathies, PE sessions will be spaced out a minimum of 48 hours apart during the Intensive Treatment Phase (the first 3 weeks).~~	This study will evaluate the effects of PE with Albutein 5% in treating ALS. PE involves a non-selective removal of plasma proteins among which include various coagulation related proteins and factors. Therefore, a transient coagulopathy is an anticipated and expected side effect related to the procedure but this has not been reported to increase the overall patient risk profile. These transient changes are normally reverted in 24 hours following the procedure. During the intensive phase of the study the treatment regimen is of 2 PE procedures per week spaced out by a minimum of 48h apart to ensure the safety of each subject.	Revised the precaution with transient coagulopathy associated with PE procedures according to FDA information request and recommendations.
	PE will be carried out by a peripheral access. It is recommended that each PE be performed at a rate of 40 to 100 mL/min with a continuous flow blood cell separator. Peripheral access must be assessed at the baseline visit (V0), which consists of a subjective assessment of peripheral veins by the nursing staff of the DHMC.	PE will be preferably carried out by a peripheral venous access. It is recommended that each PE be performed at a rate of 40 to 100 mL/min with a continuous flow blood cell separator. Peripheral access must be assessed at the baseline visit (V0), which consists of a subjective assessment of peripheral veins by the nursing staff of the DHMC.	Clarification added to explain the preference of starting all subjects with peripheral venous access, but allowing the switch to central catheter.
	If there is an inabililty to achieve extraction flows greater than 45 mL/min on 2 consecutive PE sessions, the subject	If during the course of treatment phase, there is an inabililty to achieve extraction flows greater than 45	Added PE discharge criteria

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	will be offered the possibility of installing a central venous access line. Subjects who do not consent to the central venous access lines when indicated will be withdrawn from the study. Volume to be exchanged: 100 to 110% of the plasma volume based on gender, weight, and the hematocrit of the subject. PE will be carried out with a Cobe Spectra or Optia blood cell separator, using a citrate solution (ACD-A) as anticoagulant in a ratio of 1:10 and continuous infusion of calcium gluconate 1 g per hour of procedure. The approximate duration of the process will be 1.5 to 3 hours. This also includes preparation time and subsequent monitoring after the PE. The subject will remain in the DHMC facilities, being monitored by the nursing staff during the entire process~~, until the staff is confident the subject may be discharged in a stable clinical condition similar to that prior to the PE.~~	mL/min on 2 consecutive PE sessions, the subject will be offered the possibility of installing a central venous access line. Subjects who do not consent to the central venous access lines when indicated will be withdrawn from the study. Volume to be exchanged: 100 to 110% of the plasma volume based on gender, weight, and the hematocrit of the subject. PE will be carried out with a Cobe Spectra or Optia blood cell separator, using a citrate solution (ACD-A) as anticoagulant in a ratio of 1:10 and continuous infusion of calcium gluconate 1 g per hour of procedure. The approximate duration of the process will be 1.5 to 3 hours. This also includes preparation time and subsequent monitoring after the PE. The subject will remain in the DHMC facilities, being monitored by the nursing staff during the entire process per standard operating procedures of the Outpatient Apheresis Unit. Discharge criteria will include presenting stable vital signs as compared to the pre-procedure baseline vitals (any difference should be clinically relevant) as well as no new (not present prior to PE procedure) ongoing clinical signs and/or symptoms. Any subject who develops new clinical symptoms or an adverse event during PE will be evaluated by a research nurse. The final decision on subject management and discharge will be based on that clinical assessment by the research nurse in consultation with the attending apheresis physician. In addition, it is mandatory that a designated caregiver	according to the FDA information request and recommendations.

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		is present to accompany the subject once he/she leaves the study center after the PE procedure.	
Section 5.3	5.3. Permitted concomitant ~~and restricted~~ treatments ~~Permitted concomitant treatments~~ The subject may continue receiving his/her usual medication. The permitted treatments shall be those necessary from the point of view of the investigator. In particular, the administration of Riluzole may be considered at a dose of 1 tablet of 50 mg in the morning and 1 tablet of 50 mg at night (2 tablets per day, one every 12 hours). The administration of Riluzole should be avoided immediately before and after the PE. All medication administered during the study will be considered as concomitant medication and must be recorded in the case report form (CRF). ~~Restricted treatments during the study~~ ~~Any medication that may affect coagulation parameters or the PE procedure should be restricted:~~ ~~1. The transfusion of packed red blood cells in the absence of gastrointestinal bleeding and symptoms of tissue anoxia is to be performed only in subjects with a hematocrit <20%.~~ ~~2. Platelet transfusion is to be performed in cases of severe thrombocytopenia (<20,000/mm3), or in the case of bleeding complications when the platelet count is below 50,000/mm3.~~ ~~3. The administration of fresh plasma will be performed only in the case of severe coagulation alterations (prothrombin time [PT] <20%) or in the case~~	5.3 Permitted concomitant treatments The subject may continue receiving his/her usual medication. The permitted treatments shall be those necessary from the point of view of the investigator. In particular, the administration of Riluzole may be considered at a dose of 1 tablet of 50 mg in the morning and 1 tablet of 50 mg at night (2 tablets per day, one every 12 hours). The administration of Riluzole should be avoided immediately before and after the PE. All medication administered during the study will be considered as concomitant medication and must be recorded in the case report form (CRF).	Removed the restricted treatments in response the FDA information request and recommendations, as well as adaptation the standard of care from Outpatient Apheresis Unit regarding permitted concomitant treatments.

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	~~of bleeding complications with PT <40%.~~ ~~4. It is recommended that no antiplatelet/antiagregant medication be administered on the day of the PE.~~		
Section 6.2.1 Study Time and Events Table	No previous text Safety Laboratory Analyses: Deleted coagulation from V1 No previous text Safety Laboratory Analyses: Deleted Hematological (blood) from V1 No previous text No previous text No previous text b Vital signs will be monitored 10 to 15 minutes before the PE, during the PE, and 15 to 30 minutes after ~~the~~ PE. No previous text	Added pulse oximetry at PE Day Safety Laboratory Analyses: Added Coagulation at PE Day Safety Laboratory Analyses: Added fibrinogen (plasma) at V0 and PE Day Safety Laboratory Analyses: Added biochemistry + Lipid Profile (serum) at PE Day Safety Laboratory Analyses: Added serum electrolytes at V0 and PE Day Safety Laboratory Analyses: Added Serology (serum) at V0, PE Day and V6 Add Plasma retention sample at PE Day Plasma Biomarker laboratory analyses: Added Cytokine panel, Neurofilament levels, BMAA levels, and Biomarker retention samples at V1, V2, and V3 b Vital signs <u>and pulse oximetry</u> will be monitored 10 to 15 minutes before the PE, during the PE <u>(30 min after PE procedure starts)</u>, and 15 to 30 minutes after <u>each</u> PE. c <u>Procedures performed 15 to 30 minutes after each PE.</u> d. <u>Procedures performed 10 to 15 minutes before the</u>	The table and footnotes were revised to reflect the relevant changes.

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	d. ~~B~~before and ~~immediately~~ after ~~finalizing~~ the PE ~~on the evaluation visit~~.	PE, and 15 to 30 minutes after the PE.	
Section 6.2.2	– Safety Laboratory Analyses (analysis on site): o Coagulation: ~~Fibrinogen~~, PT, and activated partial thromboplastin time (aPTT) o Blood Count: ~~H~~ematocrit, hemoglobin, erythrocytes, platelets, and leucocytes o Biochemistry + Lipid Profile: alanine transaminase (ALT), aspartate transaminase (AST), creatine kinase (CK), creatinine, ferritin, glucose, protein (pre-albumin, albumin, total protein in serum)~~, electrolytes (calcium, phosphate, sodium, potassium, chloride, bicarbonate) and total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and triglycerides~~ o Serology: ~~Immunoglobulin G,~~ HIV, HCV antibodies, and HBsAg antigen	– Safety Laboratory Analyses (analysis on site): o Coagulation: prothrombin time (PT), and activated partial thromboplastin time (aPTT) o Fibrinogen o Complete Blood Count: hematocrit, hemoglobin, erythrocytes, platelets, and leucocytes o Biochemistry + 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 o Serum electrolytes: (calcium, phosphate, sodium, potassium, chloride, and bicarbonate) o Serology: HIV 1 & 2, HCV antibodies, and HBsAg antigen	Reorganized safety laboratory tests (no new tests) to allow for assessment of fibrinogen and serum electrolytes independently from coagulation and biochemistry assessments.
Section 6.2.3	The subject will remain in the ~~DHMC~~ apheresis ~~facilities~~ during the entire PE session and for an appropriate period post PE as determined by the ~~hospital staff~~ to ensure that the subject is able to return home in a clinically stable condition. Before the PE, any abnormal condition observed since the baseline visit or the previous procedure will be recorded as an AE. Concomitant medications that the subject is receiving will also be recorded. Vital signs (blood pressure, heart rate, body temperature	The subject will remain in the Outpatient Apheresis Unit during the entire PE session and for an appropriate period post PE as determined by the research nurse to ensure that the subject is able to return home in a clinically stable condition. Before the PE, any abnormal condition observed since the baseline visit or the previous procedure will be recorded as an AE. Concomitant medications that the subject is receiving will also be recorded. A complete blood count and fibrinogen levels will be	Revised in response to the FDA information request and recommendations. Added new safety

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	and respiratory rate) will be measured 10 to 15 minutes before the beginning of each PE, during the PE, and 15 to 30 minutes after the PE procedure. In the case of pertinent abnormalities, the tests will be repeated until normalization or stabilization. Adverse events (AEs) will be recorded before, during, and after each procedure.	determined 10 to 15 minutes before each PE, following the Dartmouth Donor Room policy. Vital signs (blood pressure, heart rate, body temperature and respiratory rate) and blood oxygen using a pulse oximeter will be measured 10 to 15 minutes before the beginning of each PE, during the PE (30 min after PE procedure starts), and 15 to 30 minutes after the PE procedure. In the case of pertinent abnormalities, the tests will be repeated until normalization or stabilization. Serum electrolytes (calcium, phosphate, sodium, potassium, chloride, bicarbonate) and coagulation parameters will be measured 15 to 30 minutes after each PE procedure. Plasma retention sample will be collected 10 to 15 minutes before each PE and 15 to 30 minutes after each PE procedure. Adverse events (AEs) will be recorded before, during, and after each procedure.	determinations during PE procedures to follow standard operations procedures from site and in response to FDA recommendations.
Section 6.2.4	~~A safety determination (blood count and coagulation parameters) will be made 15 to 30 minutes before PE#7 to monitor their levels after having completed the Intensive Treatment Phase.~~		Deleted as this is not needed anymore since each PE will have a blood count determination before each procedure including PE#7.
Section 6.2.9	A complete determination of the following safety parameters will be performed. – Coagulation ~~and Blood Count~~ – Biochemistry + Lipid Profile – Serology – Serology retention samples (to be stored at -80°C)	A complete determination of the following safety parameters will be performed. – Coagulation – Fibrinogen – Complete Blood Count – Biochemistry + Lipid Profile	Reorganized safety laboratory tests (no new tests) to allow for assessment of fibrinogen and serum electrolytes independently from coagulation and biochemistry assessments.

<table>
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(Underline is added to highlight new text)</th><th>Rationale:</th></tr>
<tr><td></td><td></td><td>– Serum Electrolytes
– Serology
– Serology retention samples (to be stored at -80°C)</td><td></td></tr>
<tr><td>Section 6.3.1.1</td><td>
<table>
<tr><th>Test</th><th>Extraction Tube</th><th>Blood Volume (mL)</th><th>Comments</th></tr>
<tr><td>Biochemistry + Lipid Profile</td><td>Clotted blood</td><td>4</td><td>Analysis on site</td></tr>
<tr><td>Serology</td><td>Clotted blood</td><td>9</td><td>Analysis on site</td></tr>
<tr><td>Coagulation</td><td>Citrate</td><td>3</td><td>Analysis on site</td></tr>
<tr><td>Blood Count</td><td>EDTA K3</td><td>4</td><td>Analysis on site</td></tr>
<tr><td>Immune population profile</td><td>Na-Heparin</td><td>10</td><td>Analysis on site</td></tr>
<tr><td>Serology retention sample</td><td>SST II Advance</td><td>8.5</td><td>Aliquots are stored at -80 C</td></tr>
<tr><td>Biomarkers retention sample</td><td>EDTA K2</td><td>4</td><td>Plasma processing and aliquots of 1 mL are stored at -80 C</td></tr>
<tr><td>Cytokine panel + Neurofilament analysis</td><td>EDTA K2</td><td>5</td><td>Plasma processing and 1 mL aliquots are stored at -80 C</td></tr>
<tr><td>BMAA analysis</td><td>EDTA K2</td><td>5</td><td>Blood is to be spun down with serum and stored at -80 C</td></tr>
</table>
</td><td>
<table>
<tr><th>Test</th><th>Extraction Tube</th><th>Blood Volume (mL)</th><th>Comments</th></tr>
<tr><td>Biochemistry + Lipid Profile</td><td>Clotted blood</td><td>4</td><td>Analysis on site</td></tr>
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</td><td>Reorganized safety laboratory tests (no new tests) to allow for assessment of fibrinogen and serum electrolytes independently from coagulation and biochemistry assessments.
The table was revised accordingly to reflect the relevant changes.</td></tr>
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		Cytokine panel + Neurofilament analysis / **EDTA K2** / 5 / Plasma processing and 1 mL aliquots are stored at -80 C *BMAA analysis* / **EDTA K2** / 5 / Blood is to be spun down with serum and stored at -80 C	
Section 6.3.2.1	The ALSFRS-R includes 12 questions to assess the level of self-sufficiency of subjects in various functional domains such as ~~respiratory function,~~ bulbar function, gross ~~muscle skills, and fine muscle skills~~. Aspects of nourishment, personal care, personal autonomy, and communication are also evaluated. Each task is graded on a five point scale from 0 = is not able to do to 4 = normal ability. Individual scores are totaled to produce a final result of between 0 = worst and 48 = best.	The ALSFRS-R includes 12 questions to assess the level of self-sufficiency of subjects in various functional domains such as bulbar function (questions 1-3), fine and gross motor function (questions 4-9), and respiratory function (question 10-12). Aspects of nourishment, personal care, personal autonomy, and communication are also evaluated. Each task is graded on a five point scale from 0 = is not able to do to 4 = normal ability. Individual scores are totaled to produce a final result of between 0 = worst and 48 = best.	Clarification for SAP development
Section 6.3.2.3	The questionnaire consists of 40 items grouped into 5 representative dimensions associated with quality of life: physical mobility, activities of daily living, food and drink, communication and emotional function.	The questionnaire consists of 40 items grouped into 5 representative dimensions associated with quality of life: physical mobility (questions 1-10), activities of daily living (questions 11-20), food and drink (questions 21-23), communication (questions 24-30) and emotional function (questions 31-40).	Clarification for SAP development
Appendix 1 Study Procedure Flow-Chart	No previous entry No previous entry No previous entry	Added Pulse oximetry at PE#1 through PE#27 Added Plasma Retention Sample from PE#1 through PE#27 Added Serum Electrolytes from Baseline through PE#27 Revised Coagulation and Blood Counts to only Coagulation and added collection at PE#1 through	The table and footnotes were revised to reflect the relevant changes.

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	No previous entry No previous entry a Vital signs will be monitored 10 to 15 minutes before the PE, during the PE and 15 to 30 minutes after the PE. No previous entry	PE#27 Added Fibrinogen from Baseline through PE#27 Added Blood Count from Baseline through PE#27 a Vital signs and pulse oximetry will be monitored 10 to 15 minutes before the PE, during the PE (30 min after PE procedure starts) and 15 to 30 minutes after the PE. e Samples will be taken after the corresponding PE.	

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

Protocol Code: GBI1501

Version: 2.0, dated 05 May 2016

INVESTIGATOR SIGNATURE SHEET

I agree to perform the study in accordance with the content of this Protocol Version 2.0 dated 05 May 2016 and I agree to comply with the Standards of International Conference on Harmonization Good Clinical Practices and Declaration of Helsinki as well as with the applicable legal requirements.

Principal Investigator: [redacted]**, MD**

_____

(Signature and Date)

Principal Investigator: [redacted]**, MD, PhD**

_____

(Signature and Date)

CONTACT INFORMATION

Sponsor Representative

[redacted] **PhD**
[redacted]
Grifols Bioscience Industrial Group
Bioscience Clinical & Pharmacovigilance
Av. De la Generalitat 152-158
08174 - Sant Cugat del Valles – Barcelona.
Tel: [redacted]
Fax: [redacted]
Email: [redacted]

LIST OF ABBREVIATIONS

ACD:	Acid Citrate Dextrose
AE:	Adverse Event
ALS:	Amyotrophic Lateral Sclerosis
ALSA-Q40:	Amyotrophic Lateral Sclerosis Assessment Questionnaire 40
ALS-CBS:	Amyotrophic Lateral Sclerosis – Cognitive Behavioral Screen
ALSFRS-R:	Amyotrophic Lateral Sclerosis Functional Rating Scale - Revised
ALT:	Alanine Transaminase
ApoE:	Apolipoprotein E
aPTT:	Activated Partial Thromboplastin Time
AQC:	6-aminoquinolyl-N-hydroxysuccinimidylcarbamate
AR:	Adverse Reaction
AST:	Aspartate Transaminase
BMAA:	Beta-methylamino-L-alanine
C9orf72:	Chromosome 9 Open Reading Frame 72
CK:	Creatine Kinase
CNS:	Central Nervous System
CRF:	Case Report Form
CSF:	Cerebrospinal Fluid
DHMC:	Dartmouth-Hitchcock Medical Center
ECG:	Electrocardiogram
EDTA:	Ethylenediaminetetraacetic Acid
ELISA:	Enzyme-Linked Immunosorbent Assay
EMA:	European Medicines Agency
EMG:	Electromyography
FDA:	Food and Drug Administration
FTD:	Frontotemporal Dementia
FUS:	FUS RNA-binding protein gene (Fused in Sarcoma)
FVC:	Forced Vital Capacity
HBsAg:	Hepatitis B Surface Antigen
HCV:	Hepatitis C Virus
HDL:	High-Density Lipoprotein
HIV:	Human Immunodeficiency Virus
IL-10:	Interleukin 10
IL-12p70:	Interleukin 12-p70
IL-17A:	Interleukin 17-A
IL-1β:	Interleukin 1-Beta
IL-6:	Interleukin 6
IFN-γ:	Interferon-Gamma
INR:	International Normalized Ratio
IP-10:	IFN-γ-Inducible Protein 10

IRB:	Institutional Review Board
LC:	Liquid Chromatography
LDL:	Low-Density Lipoprotein
MCP-1:	Human Monocyte Chemoattractant Protein-1
MDC:	Macrophage-Derived Chemokine
MDSC:	Myeloid-Derived Suppressor Cells
MedDRA:	Medical Dictionary for Regulatory Activities
MIP:	Macrophage Inflammatory Protein
MRI:	Magnetic Resonance Imaging
MS:	Mass Spectrophotometry
NF:	Neurofilament
NF-L:	Neurofilament Light chain protein
PBMC:	Peripheral Blood Mononuclear Cells
PE:	Plasma Exchange
pNF-H:	Phosporilated Neurofilament Heavy chain protein
PP:	Per Protocol
PT:	Prothrombin Time
RNA:	Ribonucleic acid
SAE:	Serious Adverse Event
SD:	Standard Deviation
SOD1:	Superoxide Dismutase 1
TARDBP:	TAR DNA-Binding Protein 43 gene
TGFβ:	Transforming Growth Factor-Beta
TNF-α:	Tumor Necrosis Factor-Alpha
Treg:	Regulatory T cells
WB:	Western Blot

TABLE OF CONTENTS

SIGNATURE SHEET 2
AMENDMENT 1 – SUMMARY OF CHANGES 4
INVESTIGATOR SIGNATURE SHEET 16
CONTACT INFORMATION 17
LIST OF ABBREVIATIONS 18
TABLE OF CONTENTS 20
PROTOCOL SYNOPSIS 23
1. INTRODUCTION 29
1.1. Background 29
1.2. Rationale for conducting the study 30
2. STUDY OBJECTIVES 32
2.1. Primary Objective 32
2.2. Secondary Objectives 32
2.3. Safety Objectives 32
3. INVESTIGATIONAL PLAN 33
3.1. Study design/Development phase 33
4. SELECTION OF STUDY POPULATION 33
4.1. Inclusion and Exclusion Criteria 33
4.1.1. Inclusion Criteria 33
4.1.2. Exclusion Criteria 34
4.2. Diagnosis of ALS 34
4.3. Number of subjects 35
4.4. Withdrawal criteria and stopping rules 36
4.5. Estimated duration of recruitment period 37
5. TREATMENT OF SUBJECTS 37
5.1. Study medication and doses 37
5.2. Plasma Exchange (PE) 38
5.3. Permitted concomitant treatments 38
5.4. Measurements to assess compliance 39
5.5. Management of the Investigational Product: labeling and storage 39
5.6. Rescue Medication 39
6. TRIAL CONDUCT AND RESPONSE ASSESSMENT 39
6.1. Study variables 39
6.1.1. Primary Efficacy Variables 39

6.1.2. Secondary Variables 40
6.1.3. Safety Variables 40
6.2. Study Procedures 41
6.2.1. Study Time and Events 41
6.2.2. Baseline Visit (V0) 42
6.2.3. Plasma Exchange (PE) Treatments 44
6.2.4. Evaluation Visit (V1) 44
6.2.5. Evaluation Visit (V2) 45
6.2.6. Evaluation Visit (V3) 46
6.2.7. Follow-up Visit (V4) 46
6.2.8. Follow-up Visit (V5) 46
6.2.9. Final Study Visit (V6) 47
6.3. Assessments and Evaluation of Response 48
6.3.1. Processing blood samples and CSF 48
6.3.2. ALS assessment scales 50
6.3.3. Respiratory Function Study 51
6.3.4. Motor Function Study 51
6.3.5. Determination of cytokine panel 51
6.3.6. Immune population profile 51
6.3.7. Neurofilament proteins 52
6.3.8. β-methylamino-L-alanine (BMAA) analysis 52
7. ASSESSMENT OF SAFETY 53
7.1. Adverse Events 53
7.1.1. Warnings/Precautions 53
7.1.2. Adverse Event Monitoring 54
7.1.3. Adverse Event Definitions 54
7.1.4. Reporting of Serious Adverse Events 58
8. ETHICAL ASPECTS 59
8.1. General Considerations. 59
8.2. Subject's Information Sheet and Informed Consent. 60
8.3. Content of the study budget. 60
8.4. Insurance/Indemnification. 61
9. CONFIDENTIALITY 61
10. PRACTICAL CONSIDERATIONS 61
10.1. Handling study documents. 61

10.1.1. Documents required before beginning the study:.....61
10.2. Study File.....62
10.3. Handling, processing and correction of data. 62
10.4. Identification and labeling of samples for clinical research and maintenance responsibilities 63
10.5. Amendments to the protocol. 63
10.6. Audits and inspections by health authorities.....63
10.7. Terms of publication.....64
11. STATISTICAL ANALYSIS 64
11.1. Statistical Methods.....64
11.2. Subject Population for analysis 64
11.3. Efficacy Analysis 65
11.4. Safety Analysis. 65
11.5. Determination of sample size 65
11.6. Interim Analysis.....66
11.7. Person in charge of clinical monitoring and statistical analysis.....66
12. BIBLIOGRAPHY.....67
13. APPENDICES 71
APPENDIX 1. Study Procedure Flow-Chart 72
APPENDIX 2. Declaration of Helsinki 74
APPENDIX 3. ALS Functional Rating Scale (ALSFRS-R).....81
APPENDIX 4. ALS Cognitive Behavioral Screen (ALS-CBS).....83
APPENDIX 5. ALS Assessment Questionnaire 40 (ALSA-Q40).....87

PROTOCOL SYNOPSIS

Study Title :	Pilot study to evaluate the efficacy and safety of plasma exchange with Albutein® 5% in patients with amyotrophic lateral sclerosis
Name of the Sponsor:	GRIFOLS BIOLOGICALS Inc. 5555 Valley Boulevard Los Angeles, CA 90032
Name of Investigational Product:	Albutein® 5%
Protocol ID Number:	GBI1501
Name of active ingredient:	Human Serum Albumin
Indication:	Amyotrophic Lateral Sclerosis (ALS)
Study Centers:	Single-center at Dartmouth-Hitchcock Medical Center (DHMC), Lebanon, NH.
Study Design:	Pilot, phase IIa, prospective, open-label and single-arm study
Objectives:	<u>Primary Efficacy Objective:</u> Evaluate 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%. <u>Secondary Objectives:</u> Evaluate the effects of PE using Albutein 5% on: – Cognitive dysfunction – Beta-methylamino-L-alanine (BMAA) chronic toxicity – Neurofilament levels – Systemic inflammatory response <u>Safety Objectives:</u> Evaluate the effects of PE using Albutein 5% on: – Safety and tolerability of the procedure, including adverse events (AEs), clinical laboratory testing, physical examination, and vitals signs
Methodology:	This pilot study plans to enroll 10 subjects who have a definite, possible, or probable diagnosis of ALS, according to the revised El Escorial criteria. Enrolled subjects will be treated with PE with Albutein 5% as a replacement solution during an Intensive Treatment Phase of 2 PEs per week over 3 weeks folllowed by weekly PE for 21 weeks (Maintenance Treatment Phase). A 6-month follow up will begin after the last PE.

Duration of the treatment:	Six (6) months (24 weeks) of PE treatment with Albutein 5% and 6 months follow-up after last PE.
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.
Exclusion Criteria:	1. Subjects with pre-existing clinically significant lung disease not attributable to ALS. 2. Subjects diagnosed with other neurodegenerative diseases or 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 device or 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 the 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.).
Dose of the investigational medication:	Treatment consists of a total of 27 PE with Albutein 5% as a replacement solution administered during the following treatment phases: – Intensive Treatment Phase with 2 PE per week for 3 weeks (6 PE sessions) – Maintenance Treatment Phase with 1 PE per week for 21 weeks (21 PE sessions)
Treatment modes of administration:	The PE procedure will be preferably performed via peripheral venous access for the administration of Albutein 5%, although subjects will be allowed to switch to a central catheter during the treatment phase if repetitive problems to maintain exchange rate are encountered (see section 5.2 for details).
Study variables:	Primary Efficacy Variables: – Changes from baseline in the ALSFRS-R Functional Scale (6 measurements: Weeks 0, 4, 12, 25, 36, and 48) – Changes from baseline in the FVC (6 measurements: Weeks 0, 4, 12, 25, 36, and 48) Secondary Variables: – Changes from baseline in cognitive function determined by the Amyotrophic Lateral Sclerosis – Cognitive Behavioral Screen (ALS-CBS) test (3 measurements: Weeks 0, 25, and 48) – Changes from baseline in the motor evoked potential in

	thenar and hypothenar eminence and anterior tibialis muscle determined by electromyography (EMG) (6 measurements: Weeks 0, 4, 12, 25, 36, and 48) – Evaluation of quality of life using the Amyotrophic Lateral Sclerosis Assessment Questionnaire 40 (ALSA-Q40) test. (3 measurements: Weeks 0, 25, and 48) – Changes from baseline in plasma cytokine panel and neurofilament analysis (9 measurements:Weeks 0, 4*, 12*, 24*, 36 and 48) – Changes from baseline in Cerebrospinal Fluid (CSF) cytokine panel and neurofilament analysis (3 measurements: Weeks 0, 12, and 25) – Changes from baseline in plasma BMAA levels (9 measurements: Weeks 0, 4*, 12*, 24*, 36, and 48) – Changes from baseline in CSF BMAA levels (3 measurements: Weeks 0, 12, and 25) – Changes from baseline in plasma immune population profile (4 measurements: Weeks 0, 4,12, and 48) * In those selected visits, there will be 2 measurements of biomarkers: before and after the PE procedure. <u>Safety Variables:</u> – 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 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 determined before, during, and after each PE sessionClinical laboratory testing (coagulation, blood count, biochemistry, and/or serology) at the specified visit will also be conducted
Analytical Plan/Statistical Methodology	<u>Statistical Methods</u> Unless otherwise specified, continuous variables will be described using standard statistical measurements, i.e., number of observations, mean, standard deviation (SD), median, minimum and maximum value and 1st and 3rd quartiles. All categorical variables will be summarized in frequency and percentage tables. <u>Study Populations:</u>

	– The Safety Population will include all subjects who receive any amount of the study drug. – The 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 (FVC or ALSFRS-R) at a subsequent visit. – The Per Protocol (PP) Population will include all evaluable subjects who complete the treatment without major protocol deviations which could impact the primary efficacy assessment. Sub-group analysis of study results will be also performed based on Riluzole use (yes or no) and site of onset (limb-onset or bulbar onset). As no control group is to be included, the evaluation will be conducted descriptively based on the variables mentioned above. Data processing and evaluation procedures are described in more detail in the Statistical Analysis Plan. <u>Efficacy Analysis</u> Efficacy Analysis will be carried out on the evaluable population. The primary efficacy analyses will also be performed using the PP population. All efficacy variables and changes from the baseline will be summarized by visit. For the primary efficacy variable, the ALSFRS-R Functional Scale, in addition to the overall score, values of the different functional subdomains will be summarized by visit. For the co-primary efficacy variable, changes from baseline in the FVC will be summarized by visit. Changes from baseline in EMG profile, ALS-CBS, ALSA-Q40, and different biomarkers (cytokine panel, neurofilament, BMAA, and immune population profile) will be summarized by visit. In addition, changes in the cytokine panel, neurofilament levels, and BMAA levels before and after selected PE (PE#7, PE#15, and PE#27) will be summarized and analyzed using the non-parametric Wilcoxon test for paired data. <u>Safety Analysis</u> The Safety Analysis will be based on the safety population. All AEs (irrespective of causality) will be tabulated and summarized using descriptive statistics.

	Adverse events will be coded in accordance with the Medical Dictionary for Regulatory Activities (MedDRA) AE classification (version 17.0 or higher), and will be described by preferred term and the system organ class for severity, causality, and seriousness. The proportion of subjects and PE procedures temporarily associated with at least one AE/AR will be summarized. Clinically relevant abnormal changes (according to the investigator's clinical judgement) in vital signs and any other laboratory parameter will be recorded as AEs. The same will be done with relevant abnormal changes in any such parameter during the follow-up period. Clinical laboratory parameters and changes from baseline values will be summarized by visit. Vital signs and changes from baseline values will be summarized by visit.

1. INTRODUCTION

1.1. Background

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 difficulty in breathing, and it is often accompanied by difficulty in swallowing. Death of ALS patients is usually produced by respiratory failure.(1)

ALS is a disease of unknown etiology that belongs to the group of motor neuron diseases. Within this category, ALS is the disease with the worst prognosis. Two types of ALS are predominantly distinguished by their onset: limb-onset ALS which begins with motor impairments of the extremities and a bulbar-onset ALS with motor impairments at the level of the cranial nerves.(2) Although the clinical presentation and evolution of the disease are variable, its course is inexorably progressive and 60% of patients die within 3 years. The average survival after diagnosis falls between 2 and 5 years.(3,4)

ALS has an incidence in Western countries of between 1.5 and 2.7 new cases per 100,000 persons/year and with a prevalence of between 2.7 and 7.4 per 100,000 persons.(5) There is a slightly greater incidence and prevalence in men than in women, which equalize with age.(6) Generally, ALS occurs between the ages of 40 and 70, but the susceptibility to ALS is associated with aging, with the peak incidence between the ages of 55 and 65.

The majority of ALS cases are considered to be sporadic, and the circumstances surrounding the development of the pathology are unknown. Nevertheless, patients with a first degree relative with ALS are considered to be familial cases. This situation occurs in approximately 5 to 10% of cases. Several genes related to hereditary ALS have been discovered. The most representative of these are *C9ORF72, SOD1*, *TARDBP* and *FUS*.(7) Other than origin, the clinical presentation of sporadic and familial cases is indistinguishable.

In spite of advances in the knowledge of genetics, ALS continues to be a devastating neurodegenerative disease with an uncertain pathogenesis, for which no cure exists. Clearly associated risk factors also are unknown, but it is believed that endogenous (genetic and metabolic) as well as exogenous factors (environmental and those related to lifestyle) are at play. Some of the factors that have been linked to the onset of the disease include exposure to toxins or certain chemicals (heavy metals, pesticides, herbicides, solvents, etc.), work involving physical exertion, excessive exercise, trauma and smoking, among others.(8)

Similarly, different etiologic hypotheses have been described in an attempt to explain the origin of the sporadic cases. These include atypical infections by enterovirus or retroviral factors, oxidative stress, calcium-channel antibodies, excitotoxicity mediated by a decrease in glutamate transporters, microglial inflammation, and a decrease in neurotrophic factors, etc.(9) Among pathogenic mechanisms, one of the principal focuses of interest lies with excitotoxicity and inflammation. More recently, ALS has been linked to frontotemporal dementia (FTD) and consequently more emphasis is increasingly being placed on the cognitive component of the disease as having prognostic value.(10)

On the other hand, 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). One of the objectives of this study is to identify biomarker(s) for the disease. It is considered of particular interest to study the variations that may exist in plasma and cerebrospinal fluid (CSF) during treatment with plasma exchange (PE) in subjects with ALS. Mainly, those parameters that are altered in subjects with ALS and which can be measured in the CSF and plasma, such as beta-N-methylamino-L-alanine

(BMAA), neurofilament proteins, changes in the inflammatory response of certain cytokines and innate immunity factors.

Current treatment for this disease is related to excitotoxicity control, which has limited results with regard to the prognosis.(11) The only drug approved for its treatment is Riluzole, a glutamate release inhibitor, which lengthened life expectancy by 2 to 4 months in patients with moderate functional impairment.(12-13) 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.(14)

1.2. Rationale for conducting the study

Among the clinical studies performed throughout the last 30 years, there were 3 pilot studies, which took place in the early 1980s, using plasmapheresis in a small number of ALS patients.

Plasmapheresis is a technique by which the plasma is separated from the formed blood components (e.g., red blood cells, platelets) and discarded, and the components then returned to the subject. In plasma exchange, the extracted plasma is replaced with an equal volume of 5% albumin (other colloids such as fresh frozen plasma and crystalloids have also been used) for the purpose of maintaining blood volume and osmotic balance.

In 1979, Monstad et al. carried out a plasmapheresis study in 7 patients with ALS.(15) The patients were submitted to a weekly plasmapheresis with a fixed volume replacement of 2L (no information is available on the replacement fluid) for an average period of 8.5 months. The investigators concluded that the plasmapheresis procedure was safe but it did not affect the progression of the disease. Later, Silani et al. performed an open-label study for 6 months with plasma exchange in 4 patients affected by ALS in different stages of the disease.(16) The schedule used was 8 to 12 PE cycles removing 60-70% of the patient's plasma and replacing it with fresh plasma and 0.9% saline solution (physiologic) for two consecutive days. This regimen was repeated every 15 to 30 days over a period of 4 months. In this study the authors concluded, similar to the Monstad study, that the action of the plasmapheresis did not modify the disease progression. However, a transitory improvement was observed in two patients after the first session, but this was not observed in subsequent sessions.

Lastly, in 1983 Kelleman et al. combined immunosuppression and PE in 4 patients diagnosed with ALS for a study period of between 6 and 13 months and compared the results to a clinically matched control group.(17) After an initial treatment with Azathioprine, the plasmapheresis sessions consisted in removing a minimum of 2L of plasma and replacing it with the same volume of 5% albumin, with a regimen of 3 weekly PE for 2 weeks, followed by 1 weekly PE for a total of 3 months. The results showed that the functional capacity and muscle strength decreased in the control group as well as in the group treated, although the pulmonary function remained stable in the patients treated with plasmapheresis and albumin replacement.

More recently, in 2008 a clinical case was reported involving a patient with elevated anti-phospholipid antibody titers who was diagnosed as definite ALS based on the revised El Escorial criteria.(18) The patient was simultaneously treated with 50 cc/kg/day PE for 5 days and 50 mg/day prednisolone, followed by treatment with oral prednisolone alone, and reducing the dose gradually to 10 mg/day. An improvement in the ALS symptoms was observed in this patient after 3 months with the combined PE and prednisolone treatment.

In 2013, at the ALS Multidisciplinary Unit of the Hospital Universitari de Bellvitge (Barcelona,

Spain), 2 ALS patients were treated with PE with Albutein 5% in a compassionate use program. The regimen used was 3 PEs per week during the first 2 weeks followed by 1 PE per week for the following 12 weeks. In each procedure, 110% of the plasma volume was processed. Apart from the onset of asthenia (lack of strength), both patients tolerated the treatment well, and a stabilization of symptoms measured by the amyotrophic lateral sclerosis functional rating scale - revised (ALSFRS-R) functional scale was observed in 1 of the 2 patients on 2 occasions during the treatment period.

Given the results in the above mentioned cases, the researchers felt it would be of interest to evaluate the efficacy of PE with Albutein 5% in ALS in an experimental context since it has been more than 30 years since the last publication with PE for ALS. Since then, knowledge of the disease has increased together with the reliability of tools to measure disease progression. In the same way, PE procedures are increasingly being used and are currently considered a safe medical intervention thanks to the technical improvements of the apheresis machines and the thorough management of patients within the apheresis units.

In sum, the above studies make it possible to affirm that PE procedures are well tolerated by ALS patients. Nevertheless, the above studies were not able to demonstrate efficacy due in part to the following methodological limitations: (1) they took place before a diagnostic criterion had been established and accepted for ALS (revised El Escorial Criteria); (2) heterogeneity of the study population regarding the disease stage; (3) different therapeutic regimens were employed, and the study variables assessed were not validated; (4) PE technique involved a fixed replacement volume and did not always use albumin as the replacement solution, and (5) the sample sizes were very small.

Some of the limitations in previous studies could be mitigated now by using current knowledge of the disease and the experience acquired in ALS since the previous studies were conducted. More specifically, some of the recruitment parameters used in the clinical design have been standardized (e.g., only including subjects having less than 24 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.(19,20) Examples of diseases successfully treated with PE are: Guillain-Barré syndrome (acute treatment),(21) multiple sclerosis (chronic treatment),(22) inflammatory demyelinating polyneuropathy,(23) acute inflammatory demyelinating disease of the central nervous system (CNS),(24) and other peripheral neurological syndromes.(25) In the case of neurodegenerative diseases, Instituto Grifols S.A. is currently studying the use of PE with Albutein in Alzheimer's disease.(26)

This study is based on the use of human albumin, the most abundant protein in plasma,(27) as the replacement solution. It has been shown that albumin is not simply a plasma expander; it has other important physiological functions. Albumin is the principal transporter of multiple endogenous substances and metabolites. For example, in the extracellular liquid, albumin is the principal protein binding to fatty acids. At least 7 binding sites for fatty acids have been described through studies of X-ray crystallography. Fatty acid binding site 7 (subdomain IIa) and fatty acid binding sites 3 and 4 (subdomain IIIa) are known as class I and II drug-binding loci, respectively. Locus I is the binding site for large heterocyclic and dicarboxylic substances (eicosanoids, warfarin, bilirubin) and substances such as diazepam and tryptophan. Moreover, fatty acids bind to endogenous compounds in binding site 1, such as heme, bilirubin, and prostaglandin.(28)

Furthermore, albumin also participates in regulating microvascular permeability and has antioxidant, detoxifying, antithrombotic, and anti-inflammatory activities.(29) Data obtained in animal models of acute cerebral ischemic infarction have shown that human albumin may

also be neuroprotective by reducing the volume of the infarction and cerebral edema.(30,31) A reduction in reactive oxygen species has been observed due to the neuroprotective action of albumin in Parkinson's disease models (*in vivo* and *in vitro*), where albumin reduced the toxicity caused by 6-hydroxydopamine (6-OHDA).(32) These effects have been attributed in part to albumin being a plasma antioxidant, either directly via the binding and transport of free radical neutralizers or indirectly by sequestering transition metal ions (e.g., Cu, Fe) that have pro-oxidizing activity.(33)

Therefore, 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.

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.

A current European Medicines Agency (EMA) guidance document states that the average functional decline is about 1 point per month on the ALSFRS-R in untreated subjects.(34) However, disease progression has been found to be highly variable and does not always follow a linear descent.(35,36) 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 the reasons mentioned above, we propose a pilot study with the objective of treating 10 ALS-diagnosed subjects, with PE using Albutein 5%.

2. STUDY OBJECTIVES

2.1. Primary Objective

Evaluate the disease progression using the ALSFRS-R functional scale and the FVC of subjects affected by ALS treated with PE using Albutein 5%.

2.2. Secondary Objectives

Evaluate the effects of PE using Albutein 5% on:

- Cognitive dysfunction
- BMAA chronic toxicity
- Neurofilament levels
- Systemic inflammatory response

2.3. Safety Objectives

Evaluate the effects of PE using Albutein 5% on:

- Safety and tolerability of the procedure, including AEs, clinical laboratory testing,

physical examination, and vitals signs.

3. INVESTIGATIONAL PLAN

3.1. Study design/Development phase

Development Phase	Phase IIa
Study Design	Pilot, single-center, prospective, open-label and single arm study
Randomization procedures and treatment assignment	Not applicable for an open-label study with a single treatment
Type of control	Not applicable
Masking Techniques	Not applicable
Follow-up period	Subjects will be followed up for 6 months after the final PE treatment

4. SELECTION OF STUDY POPULATION

Subjects of both genders 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.

4.1. Inclusion and Exclusion Criteria

4.1.1. Inclusion Criteria

A subject must meet all the following inclusion criteria to be eligible for participation in this study:

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 symptom 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.

4.1.2. Exclusion Criteria

A subject meeting any of the following exclusion criteria is NOT eligible for participation in this study:

1. Subjects with pre-existing clinically significant lung disease not attributable to ALS.
2. Subjects diagnosed with other neurodegenerative diseases or 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 device or 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 the 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.).

4.2. Diagnosis of ALS

Due to the variability of the early clinical symptoms of the disease and the lack of an early diagnostic biomarker, diagnosing ALS may be difficult. Frequently the symptoms are not recognized until motor function has been lost and the average delay in diagnosis is approximately one year.(3,36) Several biomarker candidates for ALS have been identified (proteins, neurophysiological and neuroimaging parameters), but none of them have been

sufficiently validated for diagnostic or prognostic purposes.(9,36) The current ALS diagnosis is fundamentally clinical and is based on the revised El Escorial criteria.(37-41) The ability to study a homogeneous population in an early stage of the disease may be difficult due to delay in diagnosis and differences in clinical presentation of the disease.

According to the revised El Escorial criteria, the ALS diagnosis consists of:

The presence of:

(A1) clinical, neurophysiological, and neuropathological signs of lower motor neuron involvement; (A2) clinical signs of upper motor neuron involvement; and (A3) symptoms and signs, confirmed by the clinical history and examination, must be progressive with subsequent involvement of different anatomical regions.

together with the absence of:

(B1) electrophysiological or pathological signs of other diseases that could explain upper motor neuron and/or lower motor neuron involvement, and (B2) neuroimaging studies with lesions that could explain the clinical signs and electrophysiological findings.

Therefore several categories of ALS diagnosis can be described:

- **Clinically definite ALS**: clinical evidence alone of upper and lower motor neuron signs in three regions.
- **Clinically probable ALS**: clinical evidence alone of upper and lower motor neuron signs in at least two regions with some upper motor neuron signs rostral to (above) the lower motor neuron signs.
- **Clinically probable–laboratory-supported ALS**: clinical signs of upper and lower motor neuron dysfunction are in only one region, or upper motor neuron signs alone in one region with lower motor neuron signs defined by electromyography criteria in at least two limbs, together with proper application of neuroimaging and clinical laboratory protocols to exclude other causes.
- **Possible ALS**: clinical signs of upper and lower motor neuron dysfunction in only one region, or upper motor neuron signs alone in two or more regions; or lower motor neuron signs rostral to upper motor neuron signs and the diagnosis of clinically probable-laboratory-supported ALS cannot be proven.

4.3. Number of subjects

The number of subjects planned to be included in this study is 10 subjects completing the planned treatment. There has been no formal calculation for the sample size since this is a pilot study.

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.(42)

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 (primary variable of this study).(34) Thus, it may be assumed that 10 treated 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.

4.4. Withdrawal criteria and stopping rules

Subjects may withdraw or be withdrawn from the study for any of the following reasons:

1. At subjects' own request.
2. If, in the Investigator's opinion, continuation in the study would be detrimental to the subjects' well-being.
3. At the specific request of the sponsor.
4. Development of clotting or bleeding disorders due to a decrease in platelets and/or coagulation factors as a result of PE.
5. Non-compliance with inclusion or exclusion criteria.
6. Subjects with an occurrence of a concomitant disease or any medical condition which, either because of its severity or duration or necessary change in treatment, contravenes the condition of the study or puts the subject at unnecessary risk or harm.
7. Subjects with an occurrence of an AE which in the opinion of the Investigator and/or subject requires termination of treatment.
8. Subjects who are noncompliant with the protocol.
9. Subjects who develop an infection with hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV), or human Immunodeficiency virus (HIV 1 & 2) during the study. Though subjects will not be routinely tested for these pathogens during the trial, they will be under a physician's supervision throughout the study. A subject should be withdrawn if, in the opinion of his/her physician, he/she develops any of these viral infections.

The cause for the withdrawal will be documented. A final visit should be set up to obtain blood samples from the subject and for the subject to complete the final visit testings and assessments. Once the withdrawal is completed, subjects may not be readmitted to the study.

Under the following circumstances the study team will recommend to halt the trial temporarily (until modifications to the protocol are completed) or wholly (abandon all study activities):

- Temporarily halt the trial if more than 30% of subjects undergoing plasma exchange procedures have an SAE as defined as related to the Investigational Product (remember that if causal relationship of the SAE is labeled as "definite", "probable", "possible" or "doubtful/unlikely", the event will be defined as a suspected adverse drug reaction) and not attributable to the therapeutic procedure or technique of application, as judged by the principal investigator and Sponsor, requiring that both parties agree.
- Completely stop the trial if more than 50% of subjects undergoing plasma exchange procedures have an SAE as defined as related to the Investigational Product (remember that if causal relationship of the SAE is labeled as "definite", "probable", "possible" or "doubtful/unlikely", the event will be defined as a suspected adverse drug reaction) and not attributable to the therapeutic procedure or technique of application, as judged by the principal investigator and Sponsor, requiring that both parties agree.

4.5. Estimated duration of recruitment period

The recruitment period is estimated to take approximately 6 months in order to include 10 subjects.

5. TREATMENT OF SUBJECTS

5.1. Study medication and doses

Plasma exchange (PE) will be performed using Albutein 5% as the replacement solution. The main characteristics of Albutein 5% (pharmacology, preclinical safety, pharmacokinetics, and preliminary clinical data) are outlined in the package insert.

Albutein is manufactured from human plasma. Monitoring performed at the plasma collection centers and in the fractionation facility and validated procedures for infectious agent inactivation/removal included in the production process are designed to minimize the risk of the transmission of infection. However, when dealing with blood products, one cannot totally exclude the appearance of diseases due to the transmission of known and/or unknown infectious agents.

Commercial Name: Albutein 5% solution for perfusion.

Pharmaceutical Form: 250 and 500cc bottles.

Route of Administration: intravenous.

Therapeutic Group: Plasma substitutes and plasma protein fractions.

ATC Code: B05AA01

The dose of Albutein 5% for replacement following plasma removal will be calculated based on gender, weight, and the hematocrit of the subject.

Treatment Regimen

There are 2 different treatment phases in a period of 6 months (24 weeks):

- Intensive Treatment Phase: 6 PE sessions performed over 3 weeks (2 per week)
- Maintenance Treatment Phase: 21 PE sessions will be performed over 21 weeks (1 per week)

	Intensive Treatment Phase			**Maintenance Treatment Phase**																			
Weeks	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24

The treatment time was determined based on the natural progression of ALS and according to expert recommendations that a minimum period of 3 to 6 months is necessary to observe clinically relevant changes based on the ALSFRS-R. Additionally, the positive tolerability results for PE in 2 subjects treated under compassionate use for 3 months suggested that a longer period of treatment might be beneficial. Therefore, treatment duration of 6 months is

selected for the purpose of increasing the probability of observing a treatment effect.

5.2. Plasma Exchange (PE)

This study will evaluate the effects of PE with Albutein 5% in treating ALS.

PE involves a non-selective removal of plasma proteins among which include various coagulation related proteins and factors. Therefore, a transient coagulopathy is an anticipated and expected side effect related to the procedure but this has not been reported to increase the overall patient risk profile.

These transient changes are normally reverted in 24 hours following the procedure. During the intensive phase of the study the treatment regimen is of 2 PE procedures per week spaced out by a minimum of 48h apart to ensure the safety of each subject.

PE will be preferably carried out by a peripheral venous access. It is recommended that each PE be performed at a rate of 40 to 100 mL/min with a continuous flow blood cell separator.

Peripheral access must be assessed at the baseline visit (V0), which consists of a subjective assessment of peripheral veins by the nursing staff of the DHMC.

If during the course of treatment phase there is an inabililty to achieve extraction flows greater than 45 mL/min on 2 consecutive PE sessions, the subject will be offered the possibility of installing a central venous access line. Subjects who do not consent to the central venous access lines when indicated will be withdrawn from the study.

Volume to be exchanged: 100 to 110% of the plasma volume based on gender, weight, and the hematocrit of the subject.

PE will be carried out with a Cobe Spectra or Optia blood cell separator, using a citrate solution (ACD-A) as anticoagulant in a ratio of 1:10 and continous infusion of calcium gluconate 1 g per hour of procedure.

The approximate duration of the process will be 1.5 to 3 hours. This also includes preparation time and subsequent monitoring after the PE. The subject will remain in the DHMC facilities, being monitored by the nursing staff during the entire process per standard operating procedures of the Outpatient Apheresis Unit.

Discharge criteria will include presenting stable vitals signs as compared to the pre-procedure baseline vitals (any difference should be clinically relevant) as well as no new (not present prior to PE procedure) ongoing clinical signs and/or symptoms. Any subject who develops new clinical symptoms or an adverse event during PE will be evaluated by a research nurse. The final decision on subject management and discharge will be based on that clinical assessment by the research nurse in consultation with the attending apheresis physician.

In addition, it is mandatory that a designated caregiver is present to accompany the subject once he/she leaves the study center after the PE procedure.

Replacement Solution: Albutein 5% will be used in accordance with the instructions from the package insert.

5.3. Permitted concomitant treatments

The subject may continue receiving his/her usual medication.

The permitted treatments shall be those necessary from the point of view of the investigator.

In particular, the administration of Riluzole may be considered at a dose of 1 tablet of 50 mg in the morning and 1 tablet of 50 mg at night (2 tablets per day, one every 12 hours). The administration of Riluzole should be avoided immediately before and after the PE.

All medication administered during the study will be considered as concomitant medication and must be recorded in the case report form (CRF).

5.4. Measurements to assess compliance

Not applicable in this study since PE will always be administered under hospital regimen.

5.5. Management of the Investigational Product: labeling and storage

The Albutein 5% supplied is for exclusive use in the study. All unused material must be returned to the Sponsor or destroyed according to local SOPs. If the latter, a Certificate of Destruction must be provided to the Sponsor, and a copy kept in the Regulatory Binder.

The Principal Investigator (or the pharmacist) will be responsible for maintaining all the records regarding the medication used. The Sponsor will provide specific forms which will be filled out at the time medication is dispensed by the investigator, the pharmacist, or by any designated member of his/her team. These forms may be substituted by the center's own forms, if they comply with the requirements of the Sponsor.

Once the forms have been filled out, they should be signed and dated by the responsible party or by the investigator to confirm their accuracy.

The labeling must comply with the current law and storage will be carried out in the pharmacy service of DHMC.

The product should be kept at a temperature not to exceed 30°C. The product must not be used after the expiration date shown on the label. The product should not be frozen and the unused content should be discarded. Normally, the solution is clear or slightly opalescent. Do not use if the solution is turbid or any particulate matter is visualized. If large amounts are to be administered, the product should be brought to room temperature before use.

5.6. Rescue Medication

There is no rescue medication available for ALS. The Principal Investigator will be responsible for monitoring the medical needs of the subject. During the treatment phases, the investigator responsible for the PE sessions will propose the proper treatment measures based on the state of the subject's health, as necessary.

6. TRIAL CONDUCT AND RESPONSE ASSESSMENT

6.1. Study variables

6.1.1. Primary Efficacy Variables

- Changes from baseline in the ALSFRS-R functional scale (6 measurements: Weeks 0, 4, 12, 25, 36, and 48)
- Changes from baseline in the FVC (6 measurements: Weeks 0, 4, 12, 25, 36, and 48)

6.1.2. Secondary Variables

- Changes from baseline in cognitive function determined by the Amyotrophic Lateral Sclerosis – Cognitive Behavioral Screen (ALS-CBS) test (3 measurements: Weeks 0, 25, and 48)
- Changes from baseline in the motor evoked potential in thenar and hypothenar eminence and anterior tibialis muscle determined by EMG (6 measurements: Weeks 0, 4, 12, 25, 36, and 48)
- Evaluation of quality of life using the Amyotrophic Lateral Sclerosis Assessment Questionnaire 40 (ALSA-Q40) test. (3 measurements: Weeks 0, 25, and 48)
- Changes from baseline in plasma cytokine panel and neurofilament analysis (9 measurements: Weeks 0, 4*, 12*, 24*, 36, and 48)
- Changes from baseline in CSF cytokine panel and neurofilament analysis (3 measurements: Weeks 0, 12, and 25)
- Changes from baseline in plasma BMAA levels (9 measurements: Weeks 0, 4*, 12*, 24*, 36, and 48)
- Changes from baseline in CSF BMAA levels (3 measurements: Weeks 0, 12, and 25)
- Changes from baseline in plasma immune population profile (4 measurements: Weeks 0, 4,12, and 48)

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

6.1.3. Safety Variables

- 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 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 session of PE, and as deemed necessary by the investigator
- Pulse oximetry measurements recorded before, during, and after each PE session
- Clinical laboratory testings (coagulation, blood count, biochemistry, and/or serology) at the specified visit will also be conducted

6.2. Study Procedures

6.2.1. Study Time and Events

	BASELINE	EVALUATION/TREATMENT				FOLLOW-UP		
VISIT	**V0**	**PE[a] Day**	**V1**	**V2**	**V3**	**V4**	**V5**	**V6**
WEEK	-2 to 0		4	12	24	25	36	48
Informed Consent	X							
Inclusion and Exclusion Criteria	X							
Demographics	X							
Clinical History	X							
Pregnancy Test (serum)	X							
Peripheral Venous Access Assessment	X							
Physical Examination	X		X	X	X	X	X	X
Neurological Examination	X		X	X		X	X	X
Vital Signs	X	X[b]				X	X	X
Pulse Oximetry		X[b]						
EMG	X		X	X		X	X	X
Pulmonary Function								
FVC	X		X	X		X	X	X
Functional Impairment Test, Cognitive, and Behavioral Scales								
ALSFRS-R	X		X	X		X	X	X
ALSA-Q40	X					X		X
ALS-CBS	X					X		X
Concomitant Medication	X	X	X	X	X	X	X	X
Adverse Events	X	X	X	X	X	X	X	X
SAFETY Laboratory Analyses								
Coagulation (plasma)	X	X[c]						X
Fibrinogen (plasma)	X	X[d]						X
Hematological (blood)	X	X						
Biochemistry+ Lipid Profile (serum)	X			X				X
Serum electrolytes	X	X[c]						X
Serology (serum)	X							X
Serology Retention Sample	X			X				X
Plasma Retention Sample		X[d]						
Plasma BIOMARKER laboratory analyses								
Cytokine panel	X		X[d]	X[d]	X[d]		X	X
Neurofilament levels	X		X[d]	X[d]	X[d]		X	X
BMAA levels	X		X[d]	X[d]	X[d]		X	X
Biomarker retention sample	X		X[d]	X[d]	X[d]		X	X
Immune population profile analysis Analysis	X		X	X				X
CSF Laboratory Analyses								
Cell Count, Glucose, Protein and Albumin levels	X			X		X		
Cytokine panel	X			X		X		
Neurofilament levels	X			X		X		
BMAA levels	X			X		X		

[a] Procedures performed on the days subjects undergo PE. There will be 27 PE sessions.

[b] Vital signs and pulse oximetry will be monitored 10 to 15 minutes before the PE, during the PE (30 min after PE procedure starts), and 15 to 30 minutes after each PE.

[c] Procedures performed 15 to 30 minutes after each PE.

[d] Procedures perfomed 10 to 15 minutes before the PE, and 15 to 30 minutes after the PE.

See APPENDIX 1. Study Procedure Flow-Chart for another representation of the tests and procedures to be performed.

6.2.2. Baseline Visit (V0)

The Baseline Visit will extend through a maximum of 3 weeks. It will include the signing of informed consent, obtaining baseline parameters to assess subject's eligibility, and implementation of the necessary evaluations to obtain baseline parameters for the study variables. Given this window of 3 weeks for the Baseline Visit, Week 0 will be defined as the week immediately prior to when the first PE is performed allowing all other visits to be subsequently scheduled.

An initial visit will take place to explain the objective of the study and to give all the information to the subject so that the informed consent may be obtained.

A peripheral venous access assessment will be performed at the DHMC facilities, and a blood draw will take place to obtain a sample sufficient to perform the required laboratory tests. Blood samples will be taken when the subject has been fasting for a minimum of 4 hours:

- Safety Laboratory Analyses (analysis on site):
    - Coagulation: prothrombin time (PT), and activated partial thromboplastin time (aPTT)
    - Fibrinogen
    - Complete Blood Count: hematocrit, hemoglobin, erythrocytes, platelets, and leucocytes
    - Biochemistry + 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, and bicarbonate
    - Serology: HIV 1 & 2, HCV antibodies, and HBsAg antigen
    - Serology Retention Sample: retention samples taken and stored at -80ºC

Additionally, a sample will be collected for baseline measurements of different biomarker parameters:

- Plasma Biomarkers (samples will be stored at -80ºC):
    - Cytokine panel: human Apolipoproteins (ApoE2, ApoE3, and ApoE4), transforming growth factor beta (TGFβ1, 2, and 3), Interferon gamma (IFN-γ), interleukins IL-1β, IL-6, IL-10, IL-12p70, IL-17A, IP-10, MCP-1, MDC, MIP-1α, MIP-1β, tumor necrosis factor alpha (TNF-α), sCD40L, and fractalkine
    - Neurofilament analysis: phosphorylated neurofilament heavy chain (pNF-H) and neurofilament light chain (NF-L)
    - BMAA analysis: BMAA, L-Serine and amino acid profile
    - Biomarker retention samples: additional samples will be stored for future biomarker research
- Immune population profile (samples will be analyzed within 4 hours of blood draw):
    - Absolute leukocyte count (ALC) staining by Western Blot (WB): CD45, CD3, CD4, CD8, CD19, CD14, CD56, and CD16

- Myeloid-derived suppressor cells (MDSC) staining by WB: CD15, CD124, lineage, CD33, HLA-DR, CD14, CD11b, and CD45
- Regulatory T cells staining by WB: CD3, CD4, CD25, CD39, CD45, CD127, CD152, and FoxP3
- Monocytes staining by WB: CD11b, CD14, CD16, CD45, CD163, FcεR1alpha, and CX3CR1

After performing the blood draw, the subject's demographic data will be recorded. In addition, vital signs will be recorded (blood pressure, body temperature, heart and respiratory rate), and a physical and neurological examination will be performed. During subsequent study visits, only abnormal conditions observed since the previous visit will be recorded, such as concomitant medications and any AEs.

The medical history other than the ALS disease will be reviewed, including any medication taken during the previous month.

A brief history of the ALS disease will be recorded in the CRF as well as prior treatments received. If possible, past assessments related to primary endpoints (ALSFRS-R and FVC) from the last 6 months prior to enrollment will be collected in order to compare the subject's previous disease progression.

At this point, a first determination of pulmonary function (FVC) will be obtained to determine whether the subject meets the criteria for inclusion.

After obtaining the laboratory results, evaluation for all the inclusion/exclusion criteria will be performed (including a serum pregnancy test for women of child-bearing potential). If the inclusion criteria are not met or if there is an applicable exclusion criterion, the subject will not be included in the study.

Next, the baseline visit will involve an EMG study of the subject performed through the motor evoked potential in the thenar and hypothenar eminence and anterior tibialis muscle.

At the same time, the ALS scales will be administered to measure functionality and progression of disability, cognition and behavior, and overall health status:

- ALSFRS-R
- ALS-CBS
- ALSA-Q40

Finally, a lumbar puncture will be performed to analyze CSF samples at the baseline time point, which must performed a minimum of 24 hours prior to the first PE. The parameters to be evaluated are:

- Standard analysis (cell count, glucose, protein, and albumin levels)
- CSF Cytokine panel: Human Apolipoproteins (ApoE2, ApoE3, and ApoE4), transforming growth factor beta (TGFβ1, 2, and 3), Interferon gamma (IFN-γ), interleukins IL-1β, IL-6, IL-10, IL-12p70, IL-17A, IP-10, MCP-1, MDC, MIP-1α, MIP-1β, TNF-α, sCD40L, and fractalkine
- CSF Neurofilament analysis: pNF-H and NF-L
- CSF BMAA analysis: BMAA, L-Serine and amino acid profile

6.2.3. Plasma Exchange (PE) Treatments

A maximum of 27 PEs will be conducted over 6 months.

In the first Intensive Treatment Phase, 2 PEs per week will take place for 3 weeks, for a total of 6 PEs. Subsequently, 1 weekly PE for 21 weeks (Maintenance Treatment Phase) will take place to complete the 6 months (24 weeks) of treatment, for a total of 27 PEs.

If there are any important laboratory parameter changes, clinical factors, or AEs that require a PE not to be performed, the actual number of PE sessions may be less than the maximum intended.

PE visits during the Intensive Treatment Phase will be scheduled ±1 day to the planned day while always maintaining a minimum of 48 hours between two consecutive PEs.

Subsequently, the PE visits during the Maintenance Treatment Phase will be scheduled ± 2 days prior to the planned day.

The subject will remain in the Outpatient Apheresis Unit during the entire PE session and for an appropriate period post PE as determined by the research nurse to ensure that the subject is able to return home in a clinically stable condition.

Before the PE, any abnormal condition observed since the baseline visit or the previous procedure will be recorded as an AE. Concomitant medications that the subject is receiving will also be recorded.

A complete blood count and fibrinogen levels will be determined 10 to 15 minutes before each PE, following the Dartmouth Donor Room policy.

Vital signs (blood pressure, heart rate, body temperature and respiratory rate) and blood oxygen using a pulse oximeter will be measured 10 to 15 minutes before the beginning of each PE, during the PE (30 min after PE procedure starts), and 15 to 30 minutes after the PE procedure. In the case of pertinent abnormalities, the tests will be repeated until normalization or stabilization.

Serum electrolytes (calcium, phosphate, sodium, potassium, chloride, bicarbonate) and coagulation parameters will be measured 15 to 30 minutes after each PE procedure.

Plasma retention sample will be collected 10 to 15 minutes before each PE and 15 to 30 minutes after each PE procedure.

Adverse events (AEs) will be recorded before, during, and after each procedure.

6.2.4. Evaluation Visit (V1)

The first Evaluation Visit will take place during week 4 once the Intensive Treatment Phase is completed. Evaluation Visit (V1) will take place on the same day but before the PE#7 procedure.

The subject will be scheduled to come to the DHMC ALS unit and 3 tests will be performed:

1. Determination of the respiratory capacity (FVC)
2. EMG Profile
3. ALSFRS-R (may be performed in parallel with the EMG)

For consistency, if possible, these tests will be performed by the same observers for the same subject for all visits.

In addition, the visit will consist of a general physical and neurological examination, vital

signs, and documentation of concomitant medication and AEs since the previous visit.

Subsequently, a fasting blood draw will be performed before the scheduled PE at the apheresis unit at DHMC.

Plasma samples will be taken 15 to 30 minutes before and after the procedure for immune population profile (See ection 6.3.6.) and analyzed on site within 4 hours from blood draw.

Plasma samples will also be taken 15 to 30 minutes before and after the procedure for the biomarker study and should be stored at -80ºC:

- Cytokine Panel (See section 6.3.5)
- Neurofilament analysis (See section 6.3.7)
- BMAA analysis (See section 6.3.8)
- Biomarker retention samples for future biomarker research

6.2.5. Evaluation Visit (V2)

The second Evaluation Visit will take place at week 12 in the middle of the Maintenance Treatment Phase. Evaluation visit (V2) will take place before the PE#15 procedure in 2 consecutive days.

Firstly, 24 hours before PE#15 procedure, a lumbar puncture will be performed to collect the samples necessary for the biomarker study and a CSF analysis. The collection of the CSF samples should take place between 8:00 and 12:00 by the neurologist team.

On the following day but before the PE#15, the subject will come to the DHMC facilities and 3 tests will be performed:

1. Determination of the respiratory capacity (FVC)
2. EMG Profile
3. ALSFRS-R (may be performed in parallel with the EMG)

When feasible, these tests should be performed by the same observer for the same subject for all visits.

In addition, the visit will consist of a general physical and neurological examination and the collection of information on concomitant medication and AEs occurring since the previous visit.

Subsequently, the subject will go to the DHMC apheresis facilities where a fasting blood draw will be taken before the scheduled PE.

A safety determination (Biochemistry + Lipid Profile) will be performed 15 to 30 minutes before PE#15. Serology retention samples will be collected and stored at -80ºC.

Plasma samples will be taken 15 to 30 minutes before and after the procedure for immune population profile (See section 6.3.6.) and analysed on site within 4 hours from blood draw.

Plasma samples will also be taken 15 to30 minutes before and after the procedure for the following biomarker tests, which should be stored at −80ºC:

- Cytokine panels (See section 6.3.5)
- Neurofilament analysis (See section 6.3.7)
- BMAA analysis (See section 6.3.8)

- Biomarker retention samples for future biomarker research

6.2.6. Evaluation Visit (V3)

The third Evaluation Visit in this study will take place during week 24 for the purpose of finalizing the treatment period. Evaluation Visit (V3) will take place on the same day but before the last scheduled PE#27.

The visit will start with a general physical examination and the collection of information on concomitant medication and AEs occurring since the previous visit.

The subject will go to DHMC facilities where a fasting blood draw will be performed before the scheduled PE.

Plasma samples will also be taken 15 to 30 minutes before and after the procedure for the biomarker study, which should be stored at -80ºC:

- Cytokine panel (See section 6.3.5)
- Neurofilament analysis (See section 6.3.7)
- BMAA analysis (See section 6.3.8)
- Biomarker retention samples for future biomarker research

A neurologic examination, ALS scales assessment, FVC and EMG do not need to be performed at this visit.

6.2.7. Follow-up Visit (V4)

Once the PE treatments have ended, a follow-up period will commence and will last for 6 months. The first 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 and the following tests will be performed:

1. Determination of the respiratory capacity (FVC)
2. EMG Profile
3. ALSFRS-R (may be performed in parallel with the EMG)
4. Quality of life scale ALSA-Q40
5. Cognitive and behavioral scale ALS-CBS

When feasible these tests should be performed by the same observers for the same subject for all visits.

In addition, the visit will consist of a general physical and neurological examination, vital signs and the collection of information on concomitant medication and AEs occurring since the previous visit.

Lastly, a lumbar puncture will be performed at the DHMC to collect samples necessary for the CSF biomarker study and a standard CSF analysis. The collection of the CSF samples should take place between 8:00 and 12:00.

6.2.8. Follow-up Visit (V5)

The second Follow-up Visit (V5) will be scheduled on week 36 (±7 days) corresponding to

the middle point of the follow-up phase.

This visit at DHMC will be initiated with a fasting blood draw for the determination of parameters for the biomarker study. These samples should be stored at -80ºC:

- Cytokine levels (See section 6.3.5)
- Neurofilament analysis (See section 6.3.7)
- BMAA analysis (See section 6.3.8)
- Biomarker retention samples for future biomarker research

Subsequently, the subject will have 3 tests performed at DHMC:

1. Determination of the respiratory capacity (FVC)
2. EMG Profile
3. ALSFRS-R (may be performed in parallel with the EMG)

When feasible these tests should be performed by the same observers for the same patient for all visits.

In addition, the visit will consist of a general physical and neurological examination, vital signs and collection of concomitant medication and AEs since the previous visit.

6.2.9. Final Study Visit (V6)

The Final Study Visit (V6) will take place during week 48 (± 7 days) to the end of study.

Initially, the subject will come to the DHMC facilities for a fasting blood draw.

A complete determination of the following safety parameters will be performed.

- Coagulation
- Fibrinogen
- Complete Blood Count
- Biochemistry + Lipid Profile
- Serum Electrolytes
- Serology
- Serology retention samples (to be stored at -80ºC)

Plasma samples will be taken for immune population profile (See section 6.3.6) and analysed on site within 4 hours from blood draw.

Plasma samples will also be taken for the biomarker study, which should be stored at -80ºC:

- Cytokine panel (See section 6.3.5)
- Neurofilament analysis (See section 6.3.7)
- BMAA analysis (See section 6.3.8)
- Biomarker retention samples for future biomarker research

Subsequently, the subject will go to DHMC facilities where the following tests will be performed:

1. Determination of the respiratory capacity (FVC)

2. EMG Profile
3. ALSFRS-R (may be performed in parallel with the EMG)
4. Quality of life scale ALSA-Q40
5. Cognitive and behavioral scale ALS-CBS

When feasible, these tests should be performed by the same observers for the same subject for all visits.

In addition, the visit will consist of a general physical and neurological examination, vital signs and collection of information on concomitant medications and AEs since the previous visit.

6.3. Assessments and Evaluation of Response

The initial clinical evaluation will be conducted according to the standard practice. The investigator will perform a general examination of vital signs (blood pressure, heart rate, body temperature and respiratory rate) and complete physical examination during the initial visit, evaluation visits during treatment, and the final follow-up visit. Vital signs are to be measured according to standard clinical practice.

6.3.1. Processing blood samples and CSF

6.3.1.1. Blood Samples

Blood samples will be drawn at DHMC apheresis unit and collected and processed according to standard practice.

Blood samples to determine study parameters for biomarkers will be analyzed in research laboratories at local laboratory (DartLab) and by collaborating investigator Dr. Paul Cox at the Institute for Ethnomedicine (Jackson Hole, Wyoming).

Amounts of blood/serum to be collected for each test are the following:

Test	Extraction Tube	Blood Volume (mL)	Comments
Biochemistry + Lipid Profile	**Clotted blood**	4	Analysis on site
Serum Electrolytes	**Clotted blood**	4	Analysis on site
Serology	**Clotted blood**	9	Analysis on site
Coagulation	**Citrate**	3	Analysis on site
Fibrinogen	**Citrate**	1	Analysis on site
Blood Count	**EDTA K3**	4	Analysis on site
Immune population profile	**Na-Heparin**	10	Analysis on site
Plasma retention sample	**EDTA K2**	4	Aliquots are stored at -80°C
Serology retention sample	**SST II Advance**	8.5	Aliquots are stored at -80°C

Test	Extraction Tube	Blood Volume (mL)	Comments
Biomarkers retention sample	**EDTA K2**	4	Plasma processing and aliquots of 1 mL are stored at -80ºC
Cytokine panel + Neurofilament analysis	**EDTA K2**	5	Plasma processing and 1 mL aliquots are stored at -80ºC
BMAA analysis	**EDTA K2**	5	Blood is to be spun down with serum and stored at -80ºC

6.3.1.2. CSF Samples

Three (3) lumbar punctures will be performed according to standard of care at DHMC for obtaining CSF.

The CSF samples to determine safety parameters will be processed by local laboratory (DartLab) according to standard practice.

The CSF samples to determine study parameters for biomarkers will be analyzed by local laboratory (DartLab) and by collaborating investigator Dr. Paul Cox at the Institute for Ethnomedicine (Jackson Hole, Wyoming).

The minimum recommended volume of CSF obtained from each lumbar puncture will be:

Test	Extraction Tube	Minimum CSF Volume (mL)	Comments
Standard Analysis (Cell count, glucose, total protein, and albumin levels)	**Sterile tube**	2	Analysis on site
CSF cytokine panel	**Sterile tube**	0.5	Aliquot is stored at -80ºC
CSF Neurofilament analysis	**Sterile tube**	0.5	Aliquot is stored at -80ºC
CSF BMAA analysis	**Sterile tube**	5	Aliquots are stored at -80ºC
CSF retention sample	**Sterile tube**	1	Aliquot is stored at -80ºC

6.3.1.3. Sample processing

Depending on the destination of the serum and/or plasma samples, samples should be analyzed on the same day (testing priority: coagulation, blood count, and biochemistry) or they may be stored at -80°C until analyzed (cytokine panel and BMAA).

Local DHMC hematological determinations will be performed in accordance with standard institutional practice. Before the analysis takes place, the samples may be kept at 2 to 8°C, except for the CSF samples, which will be kept at room temperature.

The plasma and CSF samples obtained for retention samples must be stored at −80ºC. In the case of the CSF, the portion destined for standard analysis must not be frozen but should be kept at room temperature and analyzed immediately. The other portions may be frozen (rejecting further analysis if >10 erythrocytes are detected) or centrifuged prior to remove any residual cells.

The methods used for blood coagulation and for renal and liver function tests are those commonly used in the clinical laboratory of DHMC.

Clinical laboratory tests should be repeated at convenient intervals when abnormal results are obtained or if the investigator believes confirmatory tests are necessary; they should be repeated until the values become normal and stable. The investigator should explain all clinically relevant abnormal results.

Blood for immune population profiling and cytokine panels should be drawn ± 2 hours from baseline time of draw due to circadian rhythms of leukocytes and cytokines.

6.3.2. ALS assessment scales

6.3.2.1. 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 has been widely used as a variable to determine the efficacy of a treatment or the progression of the disease.

The ALSFRS-R includes 12 questions to assess the level of self-sufficiency of subjects in various functional domains such as, bulbar function (questions 1-3), fine and gross motor function (questions 4-9), and respiratory function (question 10-12). Aspects of nourishment, personal care, personal autonomy, and communication are also evaluated. Each task is graded on a five point scale from 0 = is not able to do to 4 = normal ability. Individual scores are totaled to produce a final result of between 0 = worst and 48 = best.

See APPENDIX 3. ALS Functional Rating Scale (ALSFRS-R).

6.3.2.2. ALS Cognitive Behavioral 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.

See APPENDIX 4. ALS Cognitive Behavioral Screen (ALS-CBS).

6.3.2.3. ALS Assessment Questionnaire 40 (ALSA-Q40):

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 1-10), activities of daily living (questions 11-20), food and drink (questions 21-23), communication (questions 24-30) and emotional function (questions 31-40).

Each item is scored from 0 to 4 according to a gradation of symptom onset frequency (never, rarely, sometimes, often, 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.

See APPENDIX 5. ALS Assessment Questionnaire 40 (ALSA-Q40).

6.3.3. Respiratory Function Study

6.3.3.1. 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.

6.3.4. Motor Function Study

6.3.4.1. 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.

6.3.5. Determination of cytokine panel

To evaluate the role of neuro-inflammation and resident microglia in ALS subjects, levels of human ApoE (ApoE2, ApoE3, and ApoE4) and TGFβ1/2/3 in plasma and CSF will be measured by enzyme-linked immunosorbent assay (ELISA).(43,44) In addition, a human 14-plex Luminex assay for several inflammatory markers (IFN-γ, IL-1β, IL-6, IL-10, IL-12p70, IL-17A, IL-17F, IP-10, MCP-1, MDC, MIP-1α, MIP-1β, TNF-α, sCD40L, and fractalkine) will be also performed.(45)

Plasma samples will be stored in aliquots at -80^{o}C and cytokines will be assayed in a single batch after final visit (V6).

6.3.6. Immune population profile

Peripheral blood mononuclear cells (PBMC) profiles have been evaluated as a marker of disease stage and progression in ALS.(46) A significant decrease in $CD14^{+}CD16^{-}$ PBMC has been noted in ALS subjects.(47,48) PBMC have a pro-inflammatory profile in ALS, and there are fewer of these cells in the periphery in ALS.(49)

Lower amounts of regulatory T cells (Tregs) in periphery in ALS dampen the immune

response and activate cytotoxic T cells, microglia, and monocytes.(50,51) Circulating monocytes ($CD14^{+}CD16^{-}$) take up residence in the CNS and become microglia. In ALS, microglia adopt a pro-inflammatory (M1) phenotype, so do monocyte-derived macrophages that contribute to the resolution of the inflammatory response through secretion of IL-10 and chemokines (CCL17/CCL22) that recruit Tregs.(52) Tregs and myeloid-derived suppressor cells (MDSC) are both important to resolving inflammation.(53) Therefore, it is planned to characterize the immune population profile in ALS subjects assessed by flow cytometry following whole blood staining for the absolute leukocyte count (CD45, CD3, CD4, CD8, CD19, CD14, CD56, and CD16); MDSC population (CD15, CD124, lineage, CD33, HLA-DR, CD14, CD11b, and CD45); Treg population (CD3, CD4, CD25, CD39, CD45, CD127, CD152, and FoxP3) and monocytes (CD11b, CD14, CD16, CD45, CD163, FcεR1alpha, and CX3CR1).

Immune population profile plasma samples will be assayed within 4 hours of blood draw.

6.3.7. Neurofilament proteins

A histopathological hallmark of ALS is the accumulation of neurofilaments (NFs) and ubiquitinated inclusions inside motor neurons.(54-56) NFs are major structural elements of the neuronal cytoskeleton and are composed of three different polypeptides: light, medium, and heavy subunits. Levels of NFs will be measured by ELISA.

Plasma samples will be stored in aliquots at -80°C and neurofilament proteins will be assayed in a single batch after last visit (V6).

6.3.7.1. Phosphorylated Neurofilament heavy chain (pNF-H)

pNF-H is elevated in subjects with ALS, and some studies suggest that levels may change over the course of disease,(56-58) pNL-H is the potential biomarker closest to validation in the ALS field. The main body of evidence for pNL-H in CSF suggests that it could be used as a diagnostic biomarker to differentiate those who have ALS versus those who do not. Less clear is the possible role of pNL-H as a prognosis biomarker.

6.3.7.2. Neurofilament light chain (NF-L)

NF-L is a main break down product of neurodegeneration, which is considered to reflect axonal damage, and it has been reported to be variably elevated in small cross-sectional studies in blood and CSF in ALS subjects.(59,60) Moreover, immunoreactivity to plasma NF-L is informative of the stage of disease progression in ALS, and thus it has been postulated as a potential biomarker with prognostic value.(61) NF-L changes are less validated but could be informative of the effect of the treatment on disease progression.

6.3.8. β-methylamino-L-alanine (BMAA) analysis

BMAA is a non-proteinogenic amino acid produced by cyanobacteria and has been shown to be present in the brain of subjects who subsequently develop ALS.(62,63) BMAA is incorporated in place of L-serine into different neuroproteins, causing misfolding and formation of tangles. It has been demonstrated that BMAA replaces L-serine by attaching to the serine tRNA. When a peptide bond forms between BMAA and the adjacent amino acid, the resultant protein is kinked and misfolded.(64)

To assess the role of BMAA in ALS subjects, different tests will be conducted in this study

using both plasma and CSF samples.

Blood serum will be stored in aliquots at -80°C and will be analyzed using triple quadrupole LC/MS/MS for BMAA levels in a single batch after last visit (V6). In addition, complete amino acid profiles of selected samples will be performed with post-column ninhydrin derivatization in a Hitachi Amino Acid Analyzer.

CSF samples will be stored in aliquots at -80°C and will be hydrolyzed and then derivatized with AQC (6-aminoquinolyl-N-hydroxysuccinimidylcarbamate) in preparation for triple quadrupole LC/MS/MS analysis. If CSF volume permits, complete amino acid profiles of selected samples will be produced through post-column derivatization with ninhydrin using the Hitachi Amino Acid Analyzer.

6.3.8.1. BMAA levels:

Measuring levels of BMAA at baseline and over the course of treatment at each evaluation visit can identify reductions in BMAA levels, which could potentially be correlated with disease progression or a halt in disease progression. Analysis will be performed using a Thermo TSQ Quantiva triple quadrupole mass spectrometer on AQC-derivatized samples of blood serum and CSF. Where sample volume permits, complete amino acid profiles will be performed using a Hitachi amino acid analyzer set for physiological fluids (see section 6.3.8.2).

6.3.8.2. L-serine levels:

L-serine levels will be determined in CSF and plasma as a potential indicator of BMAA replacement for L-serine. Analysis will be performed using a Hitachi amino acid analyzer set for physiological fluids at the Institute for Ethnomedicine Research Center, as well as with a Thermo TSQ Quantiva triple quadrupole mass spectrometer.

7. ASSESSMENT OF SAFETY

7.1. Adverse Events

7.1.1. Warnings/Precautions

As shown in the current package insert, the most serious ARs are anaphylactic shock, heart failure, and pulmonary edema. The most common ARs are anaphylactoid type reactions.

Adverse reactions to Albutein 5% normally resolve when the infusion rate is slowed or the infusion is stopped. In case of severe reactions, the infusion is stopped and appropriate treatment initiated.

The following ARs have been identified during post approval use of human albumin, including Albutein (all strengths) in decreasing order of significance: anaphylactic shock, heart failure, pulmonary edema, hypotension, tachycardia, vomiting, urticaria, rash, headache, chills, fever, flushing, and nausea.

The expected events related to PE are:(65,66) hypocalcemia (manifested by paresthesias, headaches, visual disturbances, nausea, cramps, and chest tightness) and coagulation disorders due to dilution of coagulation factors (mainly hypofibrinogenemia, with the possibility of observing minor bleeding associated with severe thrombocytopenia). Hypotension may be experienced (accompanied by pallor, sweating, bradycardia, nausea,

vomiting, syncope, sphincter relaxation, and seizures) although the risk is minimal since an amount equivalent to that extracted is infused. It is also possible to experience allergic reactions (hives, dyspnea, wheezing, hypotension, tachycardia, flushing, and eyelid edema). Other events may have a psychological origin or be inherent to the process, for example, pain after being immobilized several hours.

7.1.2. Adverse Event Monitoring

Subjects must be carefully monitored for AEs. This monitoring includes clinical and laboratory tests and physical signs. AEs should be assessed in terms of their seriousness, severity, and causal relationship to the investigational product.

The investigator is responsible for the detection and documentation of events that comply with the criterion and definition of an AE or a serious adverse event (SAE), in accordance with that set forth in this protocol. AEs and SAEs will be documented from the time the subject signs the informed consent before the first PE and until the subject returns for the last study visit, whether the subject has completed the study or has stopped prematurely for any reason.

7.1.3. Adverse Event Definitions

7.1.3.1. Adverse Events

An AE is defined as any untoward medical occurrence in a patient or clinical investigation subject administered a medicinal product or study treatment and which does not necessarily have a causal relationship with this administration. An AE may therefore be any unfavorable and unintended sign (including any abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

Baseline manifestations of the illness itself or its logical progression directly related to the course of the illness itself are not considered to be AEs, such as muscle weakness, abnormal muscle movements (e.g., twitching, clonus, or cramps), or an abnormal loss of muscle mass or body weight, breathing difficulties, emotional lability, and difficulty speaking and/or swallowing, which are considered bulbar manifestations of the underlying disease.

7.1.3.2. Suspected Adverse Reactions/Adverse Reactions

All noxious and unintended responses to a medicinal product or study treatment related to any dose should be considered suspected ARs. The phrase “responses to a medicinal product” means that a causal relationship between a medicinal product or study treatment and an AE is at least a reasonable possibility, that is, the relationship cannot be ruled out. In the framework of this study, a suspected AR with a causal relationship of “definite” will be labeled as an AR; thus, ARs are a subset of suspected ARs. The Sponsor is responsible for assessing the suspected AR expectedness during the clinical trial.

7.1.3.3. Causality of Adverse Event

The Investigator is required to provide a causality assessment for each AE reported to the Sponsor. The Sponsor will consider the Investigator’s causality assessment. Assessment of the causal relationship to the study drug will be made according to the following classifications based on Karch FE, et al.(67)

- **Definite**: an event that follows a reasonable temporal sequence from administration of the treatment or in which the treatment level has been established in body fluids or tissues; that follows a known response pattern to the suspected treatment; and that is confirmed by improvement on stopping the treatment (dechallenge), and reappearance of the event on repeated exposure (rechallenge).
- **Probable**: an event that follows a reasonable temporal sequence from administration of the treatment; that follows a known response pattern to the suspected treatment; that is confirmed by dechallenge; and that could not be reasonably explained by the known characteristics of the subject's clinical state.
- **Possible**: an event that follows a reasonable temporal sequence from administration of the treatment that follows a known response pattern to the suspected treatment but that could have been produced by the subject's clinical state or other modes of therapy administered to the patient.
- **Doubtful/Unlikely**: an event that follows a reasonable temporal sequence from administration of the treatment; that does not follow a known response pattern to the suspected treatment; but that could not be reasonably explained by the known characteristics of the subject's clinical state.
- **Unrelated**: any event that does not meet the criteria above.

The operational tool to decide the AE causal relationship is based on algorithms by Karch et al. and Naranjo et al.(68,69) When an AE is classified, assessing causal relationship by the Investigator, as "definite", "probable," "possible" or "doubtful/unlikely," the event will be defined as a suspected AR. A suspected AR with a causal relationship of "definite" will be defined as an AR. When the causal relationship is labeled "unrelated," then it will be considered that the AE is not imputable to the study treatment and it is not a suspected AR.

In addition, when a causal relationship between the study treatment and the AE cannot be ruled out by the Investigator and/or Sponsor, it means that the AE cannot be labeled "unrelated."

For any subject, all AEs that occur at any time from the beginning of investigational product administration until the final visit of the clinical trial will be considered as treatment emergent AEs (TEAEs).

AEs occurring during the two-day infusion period (i.e., from the initiation of the investigational product infusion on the first day to the completion of the total dose of investigational product on the last day) and within 72 hours following the completion of the infusion of the total dose of investigational product on the last day, regardless of other factors that may impact a possible causal association with product administration, will be defined as infusional AEs (*i.e.*, an AE temporally associated with an infusion of the investigational product) and are a subset of TEAEs.

For AEs that occur during infusions, the infusion rate in effect at the time of onset of the AE, the time of onset of the AE, and the time of AE change materially in intensity and/or resolve will be captured and recorded on the CRF.

7.1.3.4. Intensity (Severity) of Adverse Event or Suspected Adverse Reaction

AEs and suspected ARs will be classified depending on their intensity (severity) according to the following definitions:

- **Mild:** an AE which is well tolerated by the subject, causing minimum degree of malaise

and without affecting normal activities

- **Moderate:** an AE that interferes with the subject's normal activities
- **Severe:** an AE that prevents the subject from performing their normal activities

AE and suspected AR intensity gradation must be distinguished from AE and suspected AR seriousness gradation, which is defined according to event consequence. For example, headache can be mild, moderate, or severe but unusually is serious in all these cases.

The Investigator will be responsible for assessing the AE and suspected AR intensity during the clinical trial, taking into account current criteria included in this section.

7.1.3.5. Expectedness of Adverse Event or Suspected Adverse Reaction

An AE or suspected AR is considered "unexpected" if the nature, seriousness, severity, or outcome of the reaction(s) is not consistent with the reference information. The expectedness of an AR shall be determined by the Sponsor according to the reference document (*i.e.*, package insert).

Events not listed for the particular drug under investigation in the package insert are considered "unexpected" and those listed are considered "expected." When new serious ARs are received, it is the Sponsor's responsibility to determine whether the events are "unexpected" for expedited safety reporting purposes.

The reference information regarding both the study drug and the study procedure of PE can be found in section 7.1.1 of this protocol.

7.1.3.6. Seriousness of Adverse Event or Suspected Adverse Reaction; Serious Adverse Event

An AE or suspected AR is considered "serious" if, in the view of either the Investigator or Sponsor, it results in any of the following outcomes:

1. Death
2. Life-threatening AE (life-threatening in the definition of "serious" refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe)
3. In-patient hospitalization or prolongation of existing hospitalization

   Hospitalization is defined by an over 24 hours hospital admission unless it was pre-planned (i.e., elective or scheduled surgery arranged prior to the start of the study), or if the hospitalization admission is not directly associated with an AE (e.g., site standard of care, patient follow-up, or social hospitalization for purposes of respite care).
4. A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
5. A congenital anomaly/birth defect
6. An important medical event (important medical event in the definition of "serious" refers to those events which may not be immediately life-threatening, or result in death, or hospitalization but from medical and scientific judgment may jeopardize the subject and/or may require medical or surgical intervention to prevent one of the other outcomes listed above)

This definition permits either the Sponsor or the Investigator to decide whether an event is “serious.” If either the Sponsor or the Investigator believes that the event is serious, the event must be considered “serious” and evaluated by the Sponsor for expedited reporting.

A distinction should be drawn between serious and severe AEs. The term “severe” is used to describe the intensity (severity) of a specific event; the event itself, however, may be of relative minor medical significance (such as severe headache). This is not the same as “serious,” which is defined on subject/event outcome or action criteria usually associated with events that pose a threat to a subject’s life or functioning.

According to the medical criteria, an AE or a suspected AR can be classified as serious, although it does not fulfill the conditions fixed in this section if it is considered important from a medical point of view.

7.1.3.7 Adverse Event Documentation

All AEs and SAEs occurring from the subject’s **signing of the informed consent form (ICF**) until last day of subject´s participation in clinical trial must be fully recorded in the subject’s CRF or/and SAE form, respectively. If no AE has occurred during the study period, this should also be indicated in the CRF.

It is responsibility of the Investigator to ensure that AEs are appropriately recorded.

At each visit, AEs will be elicited by asking the individual a non-leading question such as “Do you feel different in way since the last visit?” Moreover, AEs will also be collected through directly observed events or spontaneously volunteered by the subject. Clearly related signs, symptoms, and abnormal diagnostic procedures should preferably be grouped together and recorded as a single diagnosis or syndrome wherever possible.

The following variables must be recorded on the AE CRF entry:

1. The verbatim term (a diagnosis is preferred)
2. Date/time of onset
3. Date/time of resolution
4. Severity (mild, moderate, severe)
5. Causality (unrelated, doubtful/unlikely, possible, probable, definite)*
6. Seriousness (yes, no)
7. Action taken (with regard to investigational product)
8. Other action (to treat the event)
9. Outcome and sequel (follow-up on AE)

**Causality assessment will be only made when the AE occurs after the subject has received the study treatment. AE occurring before subject's exposure to investigational product will be always labeled as "unrelated."*

In addition to the Investigator’s own description of the AEs, each AE will be encoded by the Sponsor according to the Medical Dictionary for Regulatory Activities (MedDRA®).

7.1.3.8 Laboratory tests and other physical evaluation abnormalities

A laboratory test abnormality considered clinically relevant, e.g., causing the subject to withdraw from the study, requiring treatment or intervention, causing apparent clinical manifestations, or judged relevant by the investigator, should be reported as an AE. Each

event must be described in detail along with start and stop dates, severity, relationship to investigational product, action taken, and outcome. Each event must be adequately supported by documentation as it appears in the subject's medical or case file.

Other abnormal test (e.g., neurological, physical examinations, functional tests) that worsen from the start and are considered clinically relevant, they should also be recorded as an AE.

The investigator shall apply their medical criteria on deciding whether the abnormal findings are important medical events as defined in section 7.1.3.6.

7.1.3.9 Type and Duration of the Follow-Up of Subjects after Adverse Events

In so far as is possible, all individuals will be followed up until the AE or suspected AR has been resolved. If an AE/suspected AR/SAE is present when the subject has completed the study, the course of the event must be followed until the final outcome is known or the event has been stabilized and no further change is expected and the Investigator decides that no further follow-up is necessary.

7.1.4. Reporting of Serious Adverse Events

7.1.4.1 Reporting Serious Adverse Event

Any SAE (see section 7.1.3.6) that occurs after **signing the study ICF through Week 48 (*i.e.*, end of study)** must be expeditiously reported whether or not considered attributable to the study drug or study procedure. Each SAE must be fully recorded in the subjects CRF and SAE form.

In addition, any SAE that occurs greater than 28 days after the last dose of investigational product should be reported if the Investigator feels that the event is related to the use of investigational product.

SAEs will be reported using the designated SAE form. When the Investigator becomes aware of an SAE, she/he must submit by email/fax a completed, signed, and dated SAE Report Form **within 24 hours** to the Sponsor.

Each SAE must be followed up until resolution or stabilization. After the initial report, all relevant information for SAE follow up, and for the outcome, must also be supplied to the Sponsor in a timely manner (within 3 days from its identification or within 24 hours for relevant new information) by means of the SAE Report Form. In addition, the Sponsor (or Contract Research Organization [CRO]) may request additional information and/or reports.

All SAE Report Forms must be reported to:

Grifols Global Pharmacovigilance
Email: [redacted]
FAX (back.-up only): [redacted]
and
Send to [redacted]

When required, and according to local law and regulations, SAEs must be reported to the IRB/EC and regulatory authorities.

There may be situations in which an SAE has occurred and the investigator has minimal information to include in the initial report. In such cases it is important for the investigator to make an evaluation of the relationship for each event prior to the submission of the SAE form because this evaluation is used to determine the requirements of the reports issued to regulatory authorities. The investigator may change his opinion for the causal relationship once he/she has additional information and rectifies the SAE reports if necessary.

7.1.4.2. Reporting Pregnancy

While pregnancy itself is not a true "AE," pregnancy occurring in a clinical study must be followed to collect information regarding the experiences of gestation and pregnancy with investigational product exposure. The investigator must report any pregnancy that occurs in a study subject (subsequent to informed consent signature through end of study). Any female subject who becomes pregnant during the study will be discontinued from the study and will be followed for pregnancy outcome.

A pregnancy not verified before randomization but occurring during the course of the study will be not considered an AE unless a relation to the study drug is suspected. In any case, a *Pregnancy Report Form* must be completed and submitted to the Sponsor as soon as possible. A copy of the Form should be filed at the study site for follow-up until the end of the pregnancy.

For any pregnancy with an outcome of live birth, the newborn infant will be followed until one month of age. Any anomalies, complications, abnormal outcomes, or birth defect observed in the child must be reported as an SAE within 24 hours of the investigator's or study personnel's first knowledge.

8. ETHICAL ASPECTS

8.1. General Considerations.

The procedures set out in this protocol, pertaining to the conduct, evaluation, and documentation of this study, are designed to ensure that the Sponsor and Investigator abide by ICH Good Clinical Practice guidelines, the Declaration of Helsinki (APPENDIX 2. Declaration of Helsinki), and will also be carried out in keeping with applicable local law(s) and regulation(s). This may include an audit by the Sponsor representatives and/or an inspection by Regulatory Authority representatives at any time. The Investigator must agree to the audit or inspection of study-related records by the Sponsor representatives and/or Regulatory Authority representatives and must allow direct access to source documents to the Sponsor and/or Regulatory Authority representatives.

Modifications to the study protocol will not be implemented by either the Sponsor or the Investigator without agreement by both parties. However, the Investigator may implement a deviation from, or a change to, the protocol to eliminate an immediate hazard(s) to the study subjects without prior IRB/Sponsor approval/favorable opinion. As soon as possible, the implemented deviation or change, the reasons for it, and, if appropriate, the proposed protocol amendment should be submitted to the IRB/Sponsor. Any deviations from the protocol must be fully explained and documented by the Investigator.

Documented approval from the appropriate IRB will be obtained prior to study start, according to ICH GCP guidelines, local laws, regulations, and organizations. When necessary, an extension, amendment, or renewal of the IRBs approval must be obtained and

also forwarded to the Sponsor. The IRB must supply to the Sponsor, upon request, a list of the IRB members involved in the vote and a statement to confirm that the IRB is organized and operates according to ICH GCP guidelines and applicable laws and regulations.

Regulatory Authority approvals/authorizations/notifications, where required, must be in place and fully documented prior to study start. Study information including contact information for Investigator sites responsible for conducting the study will be posted on a publicly accessible clinical registry(ies) as required by local law.

8.2. Subject's Information Sheet and Informed Consent.

Subject information and ICF will be provided to Investigator sites. Prior to the beginning of the study, the investigator must have the IRB/EC written approval/favorable opinion of the written ICF and any other written information to be provided to subjects. The written approval of the IRB/EC together with the approved subject information/ICF must be filed in the study files, and a copy of the documents must also be provided to Sponsor by the Investigator site.

Written ICF must be obtained before any study specific procedure takes place. Participation in the study and date of ICF given by the subject should be documented appropriately in the subject's files. A signed copy of the subject ICF will be provided to the subject or subject's authorized representative.

All subjects who are candidates for participating in the study (and their legal representative if he/she is disabled) shall be properly informed of the nature of the study, and their free and voluntary consent will be requested. The nature, purpose, and procedures of the study will be explained to each subject selected (and his/her representative), and the possible risks as a result thereof will be described.

The subject (and his/her representative) must be informed that his/her participation is voluntary and that he/she may withdraw at any time without this involving any consequence. He/she must also be informed that the Sponsor, their representatives, and the responsible authorities will have access to the clinical data.

Subjects should freely give their informed consent before inclusion in the study. The lead investigator will obtain the signed informed consent in writing from each subject or next of kin. Each subject will be given an information sheet and will be informed by the lead investigator of the benefits and risks of participating in the study and of their right to withdraw at any time. The subject will be identified with an individual code. An information sheet will be filled out by the subject which will be filed in the hospital.

The investigator must also sign and date the consent form, thus indicating that the informed consent has been obtained, that the subject (and his /her representative) has had the opportunity to ask questions, and that they have been properly answered.

The subject or legal representative will receive a copy of the information and consent form. The original consent form will be filed with the study documentation.

8.3. Content of the study budget.

A contract will be drawn up in agreement with the principal investigator, the management at the study site and the Sponsor, in which the costs for monitoring the procedures carried out in the trial, for each participant and at each visit, will be outlined. The IRB will review and approve the financial report associated with the contract.

8.4. Insurance/Indemnification.

The Sponsor will purchase an insurance policy that covers all possible damages that the subject may suffer as a result of the proper implementation of the study procedure, in accordance with applicable law, to be renewed periodically throughout the time the study is conducted.

9. CONFIDENTIALITY

All information related to the procedure, drugs, patents, scientific data and other information on materials will be considered confidential and property of the Sponsor.

However, the study protocol and other relevant documents shall be submitted to the IRB and the regulatory authorities in order to obtain approval for the clinical trial.

All records identifying the subject will be kept confidential and, to the extent permitted by the applicable laws and/or regulations, will not be made publicly available.

Subject names will not be supplied to the Sponsor. Only the subject number and subject initials will be recorded in the CRF, and if the subject's name appears on any other document (e.g., EMG report), it must be obliterated before a copy of the document is supplied to the Sponsor. Study findings stored on a computer will be stored in accordance with local data protection laws. Subjects will be informed in writing that representatives of the Sponsor, IRB, or regulatory authorities may inspect their medical records to verify the information collected, and that all personal information made available for an audit or inspection will be handled in strictest confidence and in accordance with local data protection laws.

If the results of the study are published, the subject's identity will remain confidential.

The Investigator will maintain a list to enable subjects' records to be identified, and maintain a file for each subject participating in the study, where the researcher must include all information related to the subject or the treatment. The CRF shall be on paper. The personal information for each subject in the study (age, sex, health information) is confidential, and the subject's identity is never disclosed unless necessary to meet the objectives of the trial or in a medical emergency or due to a legal requirement.

The investigator agrees that the Sponsor has the right to use the results of the clinical trial, including the CRF database, or reports with or without comments and with or without analysis to deliver to the authorities responsible for approving the license and that it is possible that they may be disclosed if necessary to other investigators. To allow the use of information obtained in the clinical trial, the investigator realizes that he/she is obliged to provide complete test results and all information developed during the study to the company.

10. PRACTICAL CONSIDERATIONS

10.1. Handling study documents.

10.1.1. Documents required before beginning the study:

1. Commitment of the investigators to participate in the clinical study following the duly signed protocol
2. Updated curriculum vitae for the investigators involved in the study
3. Approval of the IRB

4. Sample of the informed consent approved by the IRB
5. Approval of the regulatory authorities, if necessary
6. Normal laboratory values for all the tests required in the study

10.2. Study File.

The essential documents must be kept for at least 2 years from the last authorization of the procedure and until there are no pending or contemplated registrations in any country or until at least 2 years have passed since formal discontinuation of clinical development of the investigational procedure. If the local or regulatory authorities so require, these documents must be retained for a longer period of time. Before the investigator destroys material related to the clinical study, he/she must obtain approval in writing from the Sponsor.

The investigator should keep a file where the full name and address of the subject and all signed informed consents are included for at least 15 years after completion of the trial. Any original study-related information that permits verification of inclusion and exclusion criteria, including clinical history, a copy of all data collection logs, and documents on the use of the investigational product, must be stored for as long a time period as permitted by the center.

Upon request of the monitor, auditor, IRB, or regulatory authorities, the investigator shall provide direct access to all documents related to the clinical trial.

The following study documents must be filed:

1. Final version of the signed protocol and subsequent amendments
2. Approval of the IRB
3. Approval of the regulatory authorities, if necessary
4. Informed consent from each subject
5. A complete copy of the CRF and of the product distribution
6. Range of normal values for the medical techniques or laboratory procedures
7. Correspondence between the investigator and the IRB or the Sponsor
8. Monitoring visit reports
9. Clinical Study report
10. Auditing certificate (if available)
11. Serious adverse event reports to the Sponsor or the regulatory authorities

10.3. Handling, processing and correction of data.

The data shall be collected using a CRF specifically designed for the study and in a paper format.

The CRF must be filled out using black ink. If it is necessary to make any corrections, a line will be drawn through the information one wishes to correct and the correct information must be written above the corrected information (or to the side when lacking sufficient space). The person correcting the information must affix his/her initials, date, and sign it. The reason for making the correction should be specified in case it is not obvious. Correction fluid should never be used.

The investigator must date and sign the pages of the logbook when they are complete.

All the original documentation (laboratory results, treatment sheets, etc.) will be kept by the investigator. The investigator will also keep a complete copy of the CRF together with the informed consent and the other study documents for a possible future audit.

10.4. Identification and labeling of samples for clinical research and maintenance responsibilities

Albutein 5% that will be used in the clinical study will come from commercially available batches, but the labeling will be adapted to the clinical study.

The vials of Albutein 5% are not labeled for a specific subject because this study is not controlled and is not blinded. The investigator may take the amount needed for the treatment of a subject from the vials. The amount will be documented in the CRF, and the batch number and expiration of each vial received by the study subjects will also be recorded.

10.5. Amendments to the protocol.

The investigator and the Sponsor must agree to any change in the protocol after its implementation. Any amendments to the protocol must be reported so that the competent regulatory authorities may take it under consideration in accordance with current regulations. Approval of the amendment may be requested if it is deemed to be necessary.

Any attached document or document related to the present study protocol must be considered as an integral part of the protocol. After reviewing and signing the protocol, neither the investigators nor the Sponsor may make changes or alterations without the written consent of both.

If it is necessary to make an amendment or alteration to the protocol once signed, this modification shall be discussed and agreed upon between the principal investigator and the Sponsor and signed by both parties. The amendments to the protocol shall make up an integral part of the original protocol. The competent authorities and the IRB should be informed of any amendments to the protocol that may affect the safety of the subjects, the design and purpose of the test, an increase in dose, change in length of exposure to the investigational drug, increase of over 15% or more in the number of subjects treated, the addition of a new test or procedure, or non-performance of a test intended to monitor safety.

Changes in minor procedures will be carried out keeping track of document changes. This includes changes in the appendices which are not subject to IRB approval. Modifications in the contact information of the central laboratory, medical monitor, study coordinator, and/or procedures for processing biological samples only require changes in the appropriate appendices.

An administrative amendment to a study protocol includes any minor corrections or clarifications that have no significant impact on the way the clinical trial will be performed and no effect on the safety of the subject (i.e., administrative changes such as changing phone number(s), logistical changes, etc.). Any changes to the protocol (protocol amendments and administrative amendments) shall be included in an updated study protocol, with a list of all changes and justification, when necessary.

10.6. Audits and inspections by health authorities.

A person designated by the Sponsor will monitor the study to ensure that all necessary

documentation is available and that the data collected (source records) accurately reflect the CRF data. Access to the history and clinical course of the subject will be required.

When the study is completed or at another time, prior to an agreement with the investigator, an audit to guarantee the quality of the study may be conducted.

Health officials may inspect the site where the study is being conducted, as well as any laboratory used for analyses.

10.7. Terms of publication.

Sponsor is committed to honoring the principles of academic freedom while, at the same time, protecting its confidential information, the subjects, the integrity of the study, and the study documentation all in compliance with applicable law. Institution and/or Investigator recognize that, with respect to any study that is part of a multi-site study, there is a need for a coordinated approach to any publication or presentation of results from the sites. Accordingly, the Institution/Investigator shall not publish or present any results from this study to any third parties until: (1) Sponsor publishes the results; (2) Institution and/or Investigator receives written notification from Sponsor that publication of the results is no longer planned; or (3) twelve (12) months following the close of Study, whichever occurs first.

Institution and/or Investigator shall submit to Sponsor for its review a copy of any proposed publication at least thirty (30) calendar days prior to the planned date of submission for publication or presentation. Institution and Investigator shall consider in good faith all comments received from Sponsor during the review period and shall delete Sponsor's confidential information (other than study results).

If Sponsor determines that the publication contains patentable subject matter which requires protection, Sponsor may require the delay of submission for publication or presentation for an additional period of time for the purpose of filing patent applications or otherwise take measures to protect such information.

Institution and/or Investigator shall acknowledge Sponsor's support in all publications and presentations.

11. STATISTICAL ANALYSIS

11.1. Statistical Methods

Unless otherwise specified, continuous variables will be described using standard statistical measurements, i.e., number of observations, mean, standard deviation (SD), median, minimum and maximum value, and 1st and 3rd quartiles.

All categorical variables will be summarized in frequency and percentage tables.

11.2. Subject Population for analysis

- The Safety Population will include all subjects who receive any amount of the study drug.
- The 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 (FVC or ALSFRS-R) at a subsequent visit.
- The Per Protocol (PP) Population will include all evaluable subjects who complete the

treatment without major protocol deviations which could impact the primary efficacy assessment.

Sub-group analysis of study results will be also performed based on Riluzole use (yes or no) and site of onset (limb-onset or bulbar onset).

As no control group is to be included, the evaluation will be conducted descriptively based on the variables mentioned above.

Data processing and evaluation procedures are described in more detail in the Statistical Analysis Plan.

11.3. Efficacy Analysis

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

All efficacy variables and changes from the baseline will be summarized by visit.

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

For the co-primary efficacy variable, changes from baseline in the FVC will be summarized by visit.

Changes from baseline in EMG profile, ALS-CBS, ALSA-Q40, and different biomarkers (cytokine panel, neurofilament, BMAA, and immune monitoring profile) will be summarized by visit

In addition, changes from baseline in the levels of cytokine panel, neurofilament levels, and BMAA levels before and after selected PE (PE#7, PE#15, and PE#27) will be sumarized and analysed using the non-parametric Wilcoxon test for paired data.

11.4. Safety Analysis.

The Safety Analysis will be based on the safety population.

All AEs (irrespective of causality) will be tabulated and summarized using descriptive statistics.

AEs will be coded in accordance with the AE classification from the MedDRA (version 17.0 or higher), and will be described by a preferred term and the system organ class for severity, causality, and seriousness.

The proportion of subjects and PE procedures temporarily associated with at least one AE/AR will be sumarized.

Clinically relevant abnormal changes (according to the investigator's discretion) in vital signs and any other laboratory parameter shall be recorded as AEs. The same will be done with relevant abnormal changes in any such parameter during the follow-up period.

Clinical laboratory parameters and changes from baseline values will be sumarized by visit. Vital signs and changes from baseline values will be summarized by visit.

11.5. Determination of sample size

The sample size has not been calculated formally because this is a pilot study. The decision

to recruit 10 subjects was reached to obtain the information necessary to meet the objectives of the study and to verify proof of concept of the use of PE with Albutein 5% in ALS patients.

11.6. Interim Analysis

No interim analysis will be performed.

11.7. Person in charge of clinical monitoring and statistical analysis

The clinical monitoring and the statistical analysis will be performed by a CRO selected by the Sponsor for that purpose.

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13. APPENDICES

Appendix 1. Study Procedure Flow-Chart

Appendix 2. Helsinki Declaration

Appendix 3. ALS Functional Rating Scale (ALSFRS-R)

Appendix 4. ALS Cognitive Behavioral Screen (ALS-CBS)

Appendix 5. ALS Assessment Questionnaire 40 (ALSA-Q40)

APPENDIX 1. Study Procedure Flow-Chart

Month	0	1				2				3				4				5				6				7	8	9	10	11	12
Week	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25		36			48
Treatment	B	Intensive 2PE/Week				Maintenance 1PE/Week																				Follow-Up					
Plasma Exchange #		1-2	3-4	5-6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27						
Visit	VO				V1								V2												V3	V4		V5			V6
Informed Consent	X																														
Inc / Exc Criteria	X																														
Demographics/ Clinical History	X																														
Pregnancy test (serum)	X																														
Peripheral venous access assessment	X																														
Physical Exam	X				X								X												X	X		X			X
Neurol Exam	X				X								X													X		X			X
Vital Signs	X	X^a	X^a	X^a	X^a	X^a	X^a	X^a	X^a	X^a	X^a	X^a	X^a	X^a	X^a	X^a	X^a	X^a	X^a	X^a	X^a	X^a	X^a	X^a	X^a	X^a	X		X			X
Pulse Oximetry		X^a	X^a	X^a	X^a	X^a	X^a	X^a	X^a	X^a	X^a	X^a	X^a	X^a	X^a	X^a	X^a	X^a	X^a	X^a	X^a	X^a	X^a	X^a	X^a						
AE / Concomitant Medication	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X			X
ALSFRS-R	X				X^b								X^b													X		X			X
FVC	X				X^b								X^b													X		X			X
EMG	X				X^b								X^b													X		X			X
ALSA-Q40	X																									X					X
ALS-CBS	X																									X					X
Lumbar Puncture CSF Analysis	X												X^c													X					
Cytokine panel and Neurofilament levels	X				X^d								X^d												X^d			X			X
BMAA levels	X				X^d								X^d												X^d			X			X
Biomarker retention sample	X				X^d								X^d												X^d			X			X
Immune population profile	X				X								X																		X

Month	0	1				2				3				4				5				6				7	8	9	10	11	12
Week	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25		36			48
Treatment	B	Intensive 2PE/Week			Maintenance 1PE/Week																					Follow-Up					
Plasma Exchange #		1-2	3-4	5-6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27						
Visit	VO				V1								V2												V3	V4		V5			V6
Plasma Retention Sample		X[d]	X[d]	X[d]	X[d]	X[d]	X[d]	X[d]	X[d]	X[d]	X[d]	X[d]	X[d]	X[d]	X[d]	X[d]	X[d]	X[d]	X[d]	X[d]	X[d]	X[d]	X[d]	X[d]	X[d]						
Serology Retention Sample	X												X																		X
Serology	X																														X
Biochemistry	X												X																		X
Serum Electrolytes	X	X[e]	X[e]	X[e]	X[e]	X[e]	X[e]	X[e]	X[e]	X[e]	X[e]	X[e]	X[e]	X[e]	X[e]	X[e]	X[e]	X[e]	X[e]	X[e]	X[e]	X[e]	X[e]	X[e]	X[e]						X
Coagulation	X	X[e]	X[e]	X[e]	X[e]	X[e]	X[e]	X[e]	X[e]	X[e]	X[e]	X[e]	X[e]	X[e]	X[e]	X[e]	X[e]	X[e]	X[e]	X[e]	X[e]	X[e]	X[e]	X[e]	X[e]						X
Fibrinogen	X	X[d]	X[d]	X[d]	X[d]	X[d]	X[d]	X[d]	X[d]	X[d]	X[d]	X[d]	X[d]	X[d]	X[d]	X[d]	X[d]	X[d]	X[d]	X[d]	X[d]	X[d]	X[d]	X[d]	X[d]						X
Blood Count	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X						X

B = Baseline; *PE* = Plasma Exchange; Inc/Exc = Inclusion / Exclusion; AE = Adverse Event *ALSFRS-R* = Revised ALS Func ional Scale; *FVC* = Forced Vital Capacity; *ALSA Q40* = ALS Quality of Life Questionnaire; *ALS-CBS* = ALS Cognitive and Behavioral Scale and *CSF* =Cerebrospinal Fluid; BMAA = Beta-N-methylamino-L-alanine.

[a] Vital signs and pulse oximetry will be monitored 10 to 15 minutes before the PE, during the PE (30 min after PE procedure starts) and 15 to 30 minutes after the PE.
[b] The tests at DHMC will be performed on the same day as the PE, before the PE.
[c] The lumbar puncture at visit V2 will be scheduled 24 hours before the PE corresponding to PE#15.
[d] Samples will be taken before and after the corresponding PE.
[e] Samples will be taken after the corresponding PE.

APPENDIX 2. Declaration of Helsinki

WMA Declaration of Helsinki - Ethical Principles for Medical Research Involving Human Subjects

Adopted by the

18th WMA General Assembly, Helsinki, Finland, June 1964

and amended by the

29th WMA General Assembly, Tokyo, Japan, October 1975

35th WMA General Assembly, Venice, Italy, October 1983

41st WMA General Assembly, Hong Kong, September 1989

48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996

52nd WMA General Assembly, Edinburgh, Scotland, October 2000

53rd WMA General Assembly, Washington DC, USA, October 2002 (Note of Clarification added)

55th WMA General Assembly, Tokyo, Japan, October 2004 (Note of Clarification added)

59th WMA General Assembly, Seoul, Republic of Korea, October 2008

64th WMA General Assembly, Fortaleza, Brazil, October 2013

Preamble

1. The World Medical Association (WMA) has developed the Declaration of Helsinki as a statement of ethical principles for medical research involving human subjects, including research on identifiable human material and data.

   The Declaration is intended to be read as a whole and each of its constituent paragraphs should be applied with consideration of all other relevant paragraphs.

2. Consistent with the mandate of the WMA, the Declaration is addressed primarily to physicians. The WMA encourages others who are involved in medical research involving human subjects to adopt these principles.

General Principles

3. “The health of my patient will be my first consideration,” and the International Code of Medical Ethics declares that, “A physician shall act in the patient's best interest when providing medical care.”

4. It is the duty of the physician to promote and safeguard the health, well-being and rights of patients, including those who are involved in medical research. The physician's knowledge and conscience are dedicated to the fulfillment of this duty.

5. Medical progress is based on research that ultimately must include studies involving human subjects.

6. The primary purpose of medical research involving human subjects is to understand the causes, development and effects of diseases and improve preventive, diagnostic and therapeutic interventions (methods, procedures and treatments). Even the best proven interventions must be evaluated continually through research for their safety, effectiveness, efficiency, accessibility and quality.

7. Medical research is subject to ethical standards that promote and ensure respect for all human subjects and protect their health and rights.

8. While the primary purpose of medical research is to generate new knowledge, this goal can never take precedence over the rights and interests of individual research subjects.

9. It is the duty of physicians who are involved in medical research to protect the life, health, dignity, integrity, right to self-determination, privacy, and confidentiality of personal information of research subjects. The responsibility for the protection of research subjects must always rest with the physician or other health care professionals and never with the research subjects, even though they have given consent.

10. Physicians must consider the ethical, legal and regulatory norms and standards for research involving human subjects in their own countries as well as applicable international norms and standards. No national or international ethical, legal or regulatory requirement should reduce or eliminate any of the protections for research subjects set forth in this Declaration.

11. Medical research should be conducted in a manner that minimizes possible harm to the environment.

12. Medical research involving human subjects must be conducted only by individuals with the appropriate ethics and scientific education, training and qualifications. Research on patients or healthy volunteers requires the supervision of a competent and appropriately qualified physician or other health care professional.

13. Groups that are underrepresented in medical research should be provided appropriate access to participation in research.

14. Physicians who combine medical research with medical care should involve their patients in research only to the extent that this is justified by its potential preventive, diagnostic or therapeutic value and if the physician has good reason to believe that participation in the research study will not adversely affect the health of the patients who serve as research subjects.

15. Appropriate compensation and treatment for subjects who are harmed as a result of participating in research must be ensured.

Risks, Burdens and Benefits

16. In medical practice and in medical research, most interventions involve risks and burdens.

    Medical research involving human subjects may only be conducted if the importance of the objective outweighs the risks and burdens to the research subjects.

17. All medical research involving human subjects must be preceded by careful assessment of predictable risks and burdens to the individuals and groups involved in the research in comparison with foreseeable benefits to them and to other individuals or groups affected by the condition under investigation.

    Measures to minimize the risks must be implemented. The risks must be continuously monitored, assessed and documented by the researcher.

18. Physicians may not be involved in a research study involving human subjects unless they are confident that the risks have been adequately assessed and can be satisfactorily managed.

    When the risks are found to outweigh the potential benefits or when there is conclusive proof of definitive outcomes, physicians must assess whether to continue, modify or immediately stop the study.

Vulnerable Groups and Individuals

19. Some groups and individuals are particularly vulnerable and may have an increased likelihood of being wronged or of incurring additional harm.

    All vulnerable groups and individuals should receive specifically considered protection.

20. Medical research with a vulnerable group is only justified if the research is responsive to the health needs or priorities of this group and the research cannot be carried out in a non-vulnerable group. In addition, this group should stand to benefit from the knowledge, practices or interventions that result from the research.

Scientific Requirements and Research Protocols

21. Medical research involving human subjects must conform to general y accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and adequate laboratory and, as appropriate, animal experimentation. The welfare of animals used for research must be respected.

22. The design and performance of each research study involving human subjects must be clearly described and justified in a research protocol.

    The protocol should contain a statement of the ethical considerations involved and should indicate how the principles in this Declaration have been addressed. The protocol should include information regarding funding, Sponsors, institutional affiliations, potential conflicts of interest, incentives for subjects and information regarding provisions for treating and/or compensating subjects who are harmed as a consequence of participation in the research study.

    In clinical trials, the protocol must also describe appropriate arrangements for post-trial provisions.

Research Ethics Committees

23. The research protocol must be submitted for consideration, comment, guidance and approval to the concerned research ethics committee before the study begins. This committee must be transparent in its functioning, must be independent of the researcher, the Sponsor and any other undue influence and must be duly qualified. It must take into consideration the laws and regulations of the country or countries in which the research is to be performed as well as applicable international norms and standards but these must not be allowed to reduce or eliminate any of the protections for research subjects set forth in this Declaration.

    The committee must have the right to monitor ongoing studies. The researcher must provide monitoring information to the committee, especially information about any serious adverse events. No amendment to the protocol may be made without consideration and approval by the committee. After the end of the study, the researchers must submit a final report to the committee containing a summary of the study's findings and conclusions.

Privacy and Confidentiality

24. Every precaution must be taken to protect the privacy of research subjects and the confidentiality of their personal information.

Informed Consent

25. Participation by individuals capable of giving informed consent as subjects in medical research must be voluntary. Although it may be appropriate to consult family members or community leaders, no individual capable of giving informed consent may be enrolled in a research study unless he or she freely agrees.

26. In medical research involving human subjects capable of giving informed consent, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail, post-study provisions and any other relevant aspects of the study. The potential subject must be informed of the right to refuse to participate in the study or to withdraw consent to participate at any time without reprisal. Special attention should be given to the specific information needs of individual potential subjects as well as to the methods used to deliver the information.

    After ensuring that the potential subject has understood the information, the physician or another appropriately qualified individual must then seek the potential subject's freely-given informed consent, preferably in writing. If the consent cannot be expressed in writing, the non-written consent must be formally documented and witnessed.

    All medical research subjects should be given the option of being informed about the general outcome and results of the study.

27. When seeking informed consent for participation in a research study the physician must be particularly cautious if the potential subject is in a dependent relationship with the physician or may consent under duress. In such situations the informed consent must be sought by an appropriately qualified individual who is completely independent of this relationship.

28. For a potential research subject who is incapable of giving informed consent, the physician must seek informed consent from the legally authorized representative. These individuals must not be included in a research study that has no likelihood of benefit for them unless it is intended to promote the health of the group represented by the potential subject, the research cannot instead be performed with persons capable of providing informed consent, and the research entails only minimal risk and minimal burden.

29. When a potential research subject who is deemed incapable of giving informed consent is able to give assent to decisions about participation in research, the physician must seek that assent in addition to the consent of the legally authorized representative. The potential subject's dissent should be respected.

30. Research involving subjects who are physical y or mental y incapable of giving consent, for example, unconscious patients, may be done only if the physical or mental condition that prevents giving informed consent is a necessary characteristic of the research group. In such circumstances the physician must seek informed consent from the legally

authorized representative. If no such representative is available and if the research cannot be delayed, the study may proceed without informed consent provided that the specific reasons for involving subjects with a condition that renders them unable to give informed consent have been stated in the research protocol and the study has been approved by a research ethics committee. Consent to remain in the research must be obtained as soon as possible from the subject or a legally authorized representative.

31. The physician must fully inform the patient which aspects of their care are related to the research. The refusal of a patient to participate in a study or the patient's decision to withdraw from the study must never adversely affect the patient-physician relationship.

32. For medical research using identifiable human material or data, such as research on material or data contained in biobanks or similar repositories, physicians must seek informed consent for its collection, storage and/or reuse. There may be exceptional situations where consent would be impossible or impracticable to obtain for such research. In such situations the research may be done only after consideration and approval of a research ethics committee.

Use of Placebo

33. The benefits, risks, burdens and effectiveness of a new intervention must be tested against those of the best proven intervention(s), except in the following circumstances:

    - Where no proven intervention exists, the use of placebo, or no intervention, is acceptable; or
    - Where for compelling and scientifically sound methodological reasons the use of any intervention less effective than the best proven one, the use of placebo, or no intervention is necessary to determine the efficacy or safety of an intervention
    - and the patients who receive any intervention less effective than the best proven one, placebo, or no intervention will not be subject to additional risks of serious or irreversible harm as a result of not receiving the best proven intervention.
    - Extreme care must be taken to avoid abuse of this option.

Post-Trial Provisions

34. In advance of a clinical trial, Sponsors, researchers and host country governments should make provisions for post-trial access for all participants who still need an intervention identified as beneficial in the trial. This information must also be disclosed to participants during the informed consent process.

Research Registration and Publication and Dissemination of Results

35. Every research study involving human subjects must be registered in a publicly accessible database before recruitment of the first subject.

36. Researchers, authors, Sponsors, editors and publishers all have ethical obligations with regard to the publication and dissemination of the results of research. Researchers have a duty to make publicly available the results of their research on human subjects and are accountable for the completeness and accuracy of their reports. All parties should adhere to accepted guidelines for ethical reporting. Negative and inconclusive as well as positive results must be published or otherwise made publicly available. Sources of funding, institutional affiliations and conflicts of interest must be declared in the publication. Reports of research not in accordance with the principles of this Declaration should not be accepted for publication.

Unproven Interventions in Clinical Practice

37. In the treatment of an individual patient, where proven interventions do not exist or other known interventions have been ineffective, the physician, after seeking expert advice, with informed consent from the patient or a legally authorized representative, may use an unproven intervention if in the physician's judgment it offers hope of saving life, re-establishing health or alleviating suffering. This intervention should subsequently be made the object of research, designed to evaluate its safety and efficacy. In all cases, new information must be recorded and, where appropriate, made publicly available.

APPENDIX 3. ALS Functional Rating Scale (ALSFRS-R)

ALS FUNCTIONAL RATING SCALE (ALSFRS-R)		
1. Speech	4	Normal Speech processes
	3	Detectable speech with disturbances
	2	Intelligible with repeating
	1	Speech combined with nonvocal communication
	0	Loss of useful speech
2. Salivation	4	Normal
	3	Slight but definite excess of saliva in mouth; may have nighttime drooling
	2	Moderately excessive saliva; may have minimal drooling
	1	Marked excess of saliva with some drooling
	0	Marked drooling; requires constant tissue or handkerchief
3. Swallowing	4	Normal eating habits
	3	Early eating problems – occasional choking
	2	Dietary consistency changes
	1	Needs supplemental tube feeding
	0	NPO (exclusively parenteral or enteral feeding)
4. Handwriting	4	Normal
	3	Slow or sloppy; all words are legible
	2	Not all words are legible
	1	Able to grip pen but unable to write
	0	Unable to grip pen
5a. Cutting Food and Handling Utensils (patients without gastrostomy)	4	Normal
	3	Somewhat slow and clumsy, but no help needed
	2	Can cut most foods, although clumsy and slow; some help needed
	1	Food must be cut by someone, but can still feed slowly
	0	Needs to be fed
5b. Cutting Food and Handling Utensils (patients with gastrostomy)	4	Normal
	3	Clumsy but able to perform all manipulations independently
	2	Some help needed with closures and fasteners
	1	Provides minimal assistance to caregivers
	0	Unable to perform any aspect of task

6. Dressing and Hygiene	4	Normal function
	3	Independent and complete self-care with effort or decreased efficiency
	2	Intermittent assistance or substitute methods
	1	Needs attendant for self-care
	0	Total dependence
7. Turning in bed and adjusting bed clothes	4	Normal
	3	Somewhat slow and clumsy, but no help needed
	2	Can turn alone or adjust sheets, but with great difficulty
	1	Can initiate, but not turn or adjust sheets alone
	0	Helpless
8. Walking	4	Normal
	3	Early ambulation difficulties
	2	Walks with assistance
	1	Nonambulatory functional movement only
	0	No purposeful leg movement
9. Climbing Stairs	4	Normal
	3	Slow
	2	Mild unsteadiness or fatigue
	1	Needs assistance
	0	Cannot do
10. Dyspnea	4	None
	3	Occurs when walking
	2	Occurs with one or more of the following: eating, bathing, dressing
	1	Occurs at rest, difficulty breathing when either sitting or lying
	0	Significant difficulty, considering using mechanical respiratory support
11.Orthopnea	4	None
	3	Some difficulty sleeping at night due to shortness of breath, does not routinely use more than two pillows
	2	Needs extra pillow in order to sleep (more than two)
	1	Can only sleep sitting up
	0	Unable to sleep
12. Respiratory Insufficiency	4	None
	3	Intermittent use of NIPPV
	2	Continuous use of NIPPV during the night
	1	Continuous use of NIPPV during the night and day
	0	Invasive mechanical ventilation by intubation or tracheostomy

Total Score for ALSFRS-R: ____/48

APPENDIX 4. ALS Cognitive Behavioral Screen (ALS-CBS)

Patient Id: DOB/Age: Gender:

Onset Date: FVC: Education:

Onset Region: bulbar, arm, leg, trunk, respiratory (circle one)

☐Mark if patient responses were written, attach sheet

Attention

a) Commands: I am going to say some commands. Please listen carefully and then do what I say. (If patient is unable to indicate with finger, movement can be substituted with eyes, arm or other means).

Point/indicate (with your finger) to the ceiling and then to your left. # errors 0 1+

Touch your shoulder, point to the floor, and then make a fist. Score (circle) 1 0

b) Mental Addition/Language: I am going to say some phrases. I want you to tell me the number of syllables in each phrase. For example, "the table" has 3 syllables. (Repetition of each phrase is allowed once).

1. The weather is nice. (Correct response: 5) answer_____ # errors 0 1+
2. Tomorrow will be sunny. (Correct response: 7) answer___Score (circle) 1 0

(Score 0 if >20 seconds on either)

c) Eye Movements*: Saccades and Antisaccades.

# of Correct Saccades out of 8:___ /8 Score: 8/8 =1 points, ≤7/8 = 0 points

# of Correct Antisaccades out of 8:___ /8 Score: 8/8 =2 points, 7/8 = 1 points, ≤ 6/8 = 0 points

SCORE:	**_____/ 5**

***Eye Movement Instructions**

Saccades: I am going to hold my fingers up. Please keep your head straight and look at me. When I wiggle a finger, I want you to look at that finger and then look back at me (examiner should execute this eye movement themselves to demonstrate). Look at my finger by moving your eyes only, trying to keep your head still. Each time I will wiggle a finger, look at it and then back to me (Do 2-3 trials with the patient as practice). We will do that a few times. Ready? (Do 8 random trials, pause for 1-2 seconds between each trial).

Antisaccades: Good, next I am going to wiggle a finger again, but this time, I want you to look AWAY from the finger that moves. For example, if I move this finger (wiggle one) then I want you to look at the other finger, not the one that moves, ok? (Examiner should demonstrate for patient) Let's try it (do 2-3 trials). Just I ke before, try to keep you head still and just move your eyes. After each one, look back at me. Ready? (Do 8 random trials, pause for 1-2 seconds between each trial).

Concentration

I am going to say some numbers. After I say them, I want you to say them to me backwards, or in reverse order. For example, if I say 3-6, you would say 6-3. (If written, do not allow patient to write forward span. Discontinue after failure on two consecutive trials).

	Correct	Incorrect		Correct	Incorrect
2-9 (9-2):			7-8-6-4 (4-6-8-7):		
6-4 (4-6):			5-4-1-9 (9-1-4-5):		
3-7-2 (2-7-3):			8-2-5-9-3 (3-9-5-2-8)		
5-8-1 (1-8-5):			5-7-6-3-9 (9-3-6-7-5):		

Maximum Spam Correct (Enter score):	**_____/ 5**

Tracking/Monitoring

d) Months: Please say the months of the year backwards, starting with December. (circle omissions/mark repetitions & intrusions)

Dec Nov Oct Sep Aug Jul Jun May Apr Mar Feb Jan # errors:

For 0 errors enter a score of 2 for this section.

For 1 error enter a score of 1 for this section.

For 2 or more errors enter a score of 0 for this section. Score (enter):

e) Alphabet: Please say/write the alphabet for me. (mark uncorrected errors, omissions or intrusions) # errors 0 1+

A B C D E F G H I J K L M N O P Q R S T U V W X Y Z Score (circle) 1 0

f) Alternation Task: I want you to alternate between numbers and letters, starting with 1-A, and then 2-B, 3-C, and so on. Please continue from there, alternating between number- letter, number-letter, in order, without skipping any until I tell you to stop. (Errors: Any mistake in sequencing, i.e., 7-H, or 8-9). # errors 0 1 2

4-D 5-E 6-F 7-G 8-H 9-I 10-J 11-K 12-L 13-M Score (circle) 2 1 0

SCORE:	**/ 5**

Initiation and Retrieval

Say (write) as many words as you can starting with the letter F, as quickly as you can, in 1 minute. (Show patient Fluency Rules) You cannot say/write the names of people, places or numbers. Please do not say/write the same word with just a different ending, like truck, trucks. (S words can be substituted for F words). Errors: repetitions, rule violations

FLUENCY RULES: NO NAMES OF PEOPLE

NO NAMES OF PLACES NO NUMBERS

DO NOT USE SAME WORD WITH DIFFERENT ENDING

i.	ii.
iii.	iv.
v.	vi.
vii.	viii.
ix.	x.
xi.	xii.
xiii.	xiv.
xv.	xvi.
xvii.	xviii.
xix.	xx.

SCORE:	**_____ / 5**

TOTAL SCORE:	**_____ / 20**

ALS Caregiver Behavior Questionnaire

These questions pertain to possible changes in behavior that you have noticed since the onset of ALS symptoms. As best you can, consider changes that are unrelated to physical weakness. For example, question #1 asks about interest in activities. If the person can no longer play tennis but seems interested in it (i.e. talks about it, watches it on television), then you would circle 3 or no change in level of interest. If the person has always had the trait in question, please respond no change, since there has been no change seen over time.

Compared to before ALS onset, does he/she:

1. Have less interest in topic/events that used to be important to them?

☐ 3 No change ☐ 2 Small Change ☐ 1 Medium Change ☐ 0 Large Change

2. Show little emotion, or seem less responsive emotionally?

☐ 3 No change ☐ 2 Small Change ☐ 1 Medium Change ☐ 0 Large Change

3. Seem more agreeable or pleasant than in the past with fewer worries?

☐ 3 No change ☐ 2 Small Change ☐ 1 Medium Change ☐ 0 Large Change

4. Fail to think things through before acting?

☐ 3 No change ☐ 2 Small Change ☐ 1 Medium Change ☐ 0 Large Change

5. Seem more withdrawn from others but not sad?

☐ 3 No change ☐ 2 Small Change ☐ 1 Medium Change ☐ 0 Large Change

6. Get confused or distracted more easily?

☐ 3 No change ☐ 2 Small Change ☐ 1 Medium Change ☐ 0 Large Change

7. Have less ability to deal with frustration or stress?

☐ 3 No change ☐ 2 Small Change ☐ 1 Medium Change ☐ 0 Large Change

8. Seem less concerned about the feelings of others than before?

☐ 3 No change ☐ 2 Small Change ☐ 1 Medium Change ☐ 0 Large Change

9. Get angry or irritable more easily than before?

☐ 3 No change ☐ 2 Small Change ☐ 1 Medium Change ☐ 0 Large Change

10. Seem more sarcastic or childlike than before?

☐ 3 No change ☐ 2 Small Change ☐ 1 Medium Change ☐ 0 Large Change

11. Eat more or have a new preference for particular foods?

☐ 3 No change ☐ 2 Small Change ☐ 1 Medium Change ☐ 0 Large Change

12. Have more trouble changing options or adapting to new situations?

☐ 3 No change ☐ 2 Small Change ☐ 1 Medium Change ☐ 0 Large Change

13. Show less judgment or more problems making good decisions?

☐ 3 No change ☐ 2 Small Change ☐ 1 Medium Change ☐ 0 Large Change

14. Have less awareness of obvious problems or changes or deny them?

☐ 3 No change ☐ 2 Small Change ☐ 1 Medium Change ☐ 0 Large Change

15. Have new problems with language, such as saying the wrong word more?

☐ 3 No change ☐ 2 Small Change ☐ 1 Medium Change ☐ 0 Large Change

TOTAL SCORE:	**_____/ 45**

The following questions relate to current symptoms, not changes over time: Do you think your loved one:

1. Seems depressed on most days?

☐Yes ☐No

2. Seems anxious on most days?

☐Yes ☐No

3. Seems extremely fatigued on most days?

☐Yes ☐No

4. Suffers from unexpected crying or laughing spells?

☐Yes ☐No

APPENDIX 5. ALS Assessment Questionnaire 40 (ALSA-Q40)

ALSA-Q40

Please complete this questionnaire as soon as possible. If you have any difficulties filling in this questionnaire by yourself, please have someone to help you. However it is **your** responses that we are interested in.

The questionnaire consists of a number of statements about difficulties that you may have experienced **during the last 2 weeks**. There are no right or wrong answers: your first response is likely to be the most accurate for you. **Please check the box that best describes your own experience or feelings**.

Please answer every question even though some may seem very similar to others, or may not seem relevant to you.

All the information you provide is **confidential**.

The following statements all refer to difficulties that you may have had **during the last 2 weeks**.

Please indicate, by checking the appropriate box, how often the following statements have been true for you.

The following statements all refer to certain difficulties that you may have had during the last 2 weeks. Please indicate, by checking the appropriate box, how often the following statements have been true for you.

If you cannot walk at all

please check **Always/cannot walk at all.**

How often during the last 2 weeks have the following been true?

Please check **one box** for each question

	Never	Rarely	Sometimes	Often	Always / cannot walk at all
1. I have found it difficult to walk short distances, e.g. around the house.	☐	☐	☐	☐	☐
2. I have fallen over while walking.	☐	☐	☐	☐	☐
3. I have stumbled or tripped while walking.	☐	☐	☐	☐	☐
4. I have lost my balance while walking.	☐	☐	☐	☐	☐
5. I have had to concentrate while walking	☐	☐	☐	☐	☐
6. Walking has worn me out.	☐	☐	☐	☐	☐
7. I have had pains in my legs while walking.	☐	☐	☐	☐	☐
8. I have found it difficult to go up and down the stairs	☐	☐	☐	☐	☐
9. I have found it difficult to stand up.	☐	☐	☐	☐	☐
10. I have found it difficult to move from sitting in a chair to standing upright.	☐	☐	☐	☐	☐

Please make sure that you have checked **one box for each question** before going on to next page.

The following statements all refer to certain difficulties that you may have had during the last 2 weeks. Please indicate, by checking the appropriate box, how often the following statements have been true for you.

If you cannot do the activity all

please check **Always/cannot do at all.**

How often during the last 2 weeks have the following been true?

Please check **one box** for each question

	Never	Rarely	Sometimes	Often	Always / cannot do at all
11. I have had difficulty using my arms and hands.	☐	☐	☐	☐	☐
12. I have found turning and moving in bed difficult.	☐	☐	☐	☐	☐
13. I have had difficulty picking things up.	☐	☐	☐	☐	☐
14. I have had difficulty holding books or newspapers, or turning pages.	☐	☐	☐	☐	☐
15. I have had difficulty writing clearly.	☐	☐	☐	☐	☐
16. I have found it difficult to do jobs around the house.	☐	☐	☐	☐	☐
17. I have found it difficult to feed myself.	☐	☐	☐	☐	☐
18. I have had difficulty combing my hair or brushing and/or flossing my teeth.	☐	☐	☐	☐	☐
19. I have had difficulty getting dressed.	☐	☐	☐	☐	☐
20. I have had difficulty washing at the bathroom sink.	☐	☐	☐	☐	☐

Please make sure that you have checked **one box for each question** before going on to next page.

The following statements all refer to certain difficulties that you may have had during the last 2 weeks. Please indicate, by checking the appropriate box, how often the following statements have been true for you.

If you cannot to do the activity at all
please check **Always/cannot do at all.**

How often during the last 2 weeks have the following been true?

Please check **one box** for each question

	Never	Rarely	Sometimes	Often	Always / cannot do at all
21. I have had difficulty swallowing.	☐	☐	☐	☐	☐
22. I have had difficulty eating solid food.	☐	☐	☐	☐	☐
23. I have had difficulty drinking liquids.	☐	☐	☐	☐	☐
24. I have had difficulty participating in conversations.	☐	☐	☐	☐	☐
25. I have felt that my speech has not been easy to understand.	☐	☐	☐	☐	☐
26. I have stuttered or slurred my speech.	☐	☐	☐	☐	☐
27. I have had to talk very slowly.	☐	☐	☐	☐	☐
28. I have talked less than I used to do.	☐	☐	☐	☐	☐
29. I have been frustrated with my speech.	☐	☐	☐	☐	☐
30. I have felt self-conscious about my speech.	☐	☐	☐	☐	☐

Please make sure that you have checked **one box for each question** before going on to next page.

The following statements all refer to certain difficulties that you may have had during the last 2 weeks. Please indicate, by checking the appropriate box, how often the following statements have been true for you.

How often during the last 2 weeks have the following been true?

Please check **one box** for each question

	Never	Rarely	Sometimes	Often	Always
31. I have felt lonely.	☐	☐	☐	☐	☐
32. I have been bored.	☐	☐	☐	☐	☐
33. I have felt embarrassed in social situations.	☐	☐	☐	☐	☐
34. I have felt hopeless about the future.	☐	☐	☐	☐	☐
35. I have worried that I am a burden to other people.	☐	☐	☐	☐	☐
36. I have wondered why I keep going.	☐	☐	☐	☐	☐
37. I have felt angry because of the disease.	☐	☐	☐	☐	☐
38. I have felt depressed.	☐	☐	☐	☐	☐
39. I have worried about how the disease will affect me in the future.	☐	☐	☐	☐	☐
40. I have felt as if I have lost my independence.	☐	☐	☐	☐	☐

Please make sure that you have checked **one box for each question**

Thank you for completing the questionnaire