2 SYNOPSIS

Name of Sponsor / Company:	Instituto Grifols S.A.
Name of Finished Product:	Albutein® 5%
Name of Active Ingredient:	Human Albumin
Title of Study: Pilot study on the effects of plasma exchange on motor dysfunction and cognitive function in subjects with amyotrophic lateral sclerosis.	
Investigator:	Dr. Mónica Povedano Panades
Study Center:	Hospital Universitari de Bellvitge
Publication (reference): Not applicable	
Studied Period: Date of first enrollment: 07 Nov 2014 Date of last completed: 03 Jun 2016	**Phase of development:** Phase IV

Objectives:

The primary objective of the study was to evaluate the disease progression using the Amyotrofic Lateral Sclerosis Functional Rating Scale – Revised (ALSFRS-R) score and the Forced Vital Capacity (FVC) of subjects affected by Amyotrophic Lateral Sclerosis (ALS) and treated with Plasma Exchange (PE) with Albutein 5%.

The secondary objectives of the study were to evaluate the effects of PE on cognitive dysfunction, systemic inflammatory response, oxidative damage, non-directed metabolome profile, and safety and tolerability of the procedure.

Methodology:

This was a phase IV, prospective, open-label and single-arm pilot study, for a period of 12 months (6 months of PE treatment with Albutein 5% and 6 months of follow-up after last PE).

The 6 month (24 weeks) treatment period consisted in an Intensive Treatment Phase of 2 PEs per week over 3 weeks (6 PEs in total), followed by a Maintenance Treatment Phase of one weekly PE for 21 weeks (21 PEs). In total, 27 PEs were conducted during the overall treatment phase per subject.

The working hypothesis of the study stipulates that PE with albumin may change the metabolic profile in ALS patients in both plasma and cerebrospinal fluid (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 properties and detoxifying functions via the transport and elimination of known and unknown harmful compounds.

Number of Subjects (Planned and Analyzed):

Ten (10) subjects were planned; 13 subjects were enrolled and analyzed for safety and efficacy.

Diagnosis and Main Criteria for Inclusion:

Subjects of both genders, older than 18 and younger than 70 years of age, with definite,

possible, or probable diagnosis of ALS according to the El Escorial-Arlie criteria, having experienced their first ALS symptoms within 18 months before recruitment/consent, and presented a FVC>70%.

Investigational product, Dose and Mode of Administration, Batch Number:

Albutein 5%, intravenous infusion, Lot Numbers (500 mL): GBAG4MBMG2, GBAG4MBMG3, GKAG3BP002, GKAG4CP002, IBAG4GJ001, IBAG4N2003, IBAG5NL001, TBAG4DA002

Duration of treatment:

A 6 month (24 weeks) treatment period consisting in an Intensive Treatment Phase of 2 PEs per week over 3 weeks (6 PEs in total), followed by a Maintenance Treatment Phase of one weekly PE for 21 weeks (21 PEs).

Reference Therapy, Dose and Mode of Administration, Batch Number:

Not applicable

Criteria for Evaluation:

Efficacy:

Primary Efficacy Variables:

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

Secondary Efficacy Variables:

- Changes from baseline in cognitive function determined by the Amyotrophic Lateral Sclerosis – Cognitive Behavioral Screen (ALS-CBS) test (2 post-baseline measurements: Weeks 25 and 48)
- Changes from baseline in the motor evoked potential in thenar and hypothenar eminence and anterior tibialis muscle determined by electromyography (EMG) (5 post-baseline measurements: Weeks 4, 12, 25, 36, and 48)
- Changes from baseline in neurological examination (5 post-baseline measurements: Weeks 4, 12, 25, 36, and 48)
- Evaluation of quality of life using the Amyotrophic Lateral Sclerosis Assessment Questionnaire 40 (ALSA-Q40) test (2 post-baseline measurements: Weeks 25 and 48)
- Changes from baseline in plasma oxidative stress: hydroxynonenal, malondialdehyde-lysine, nitro-tyrosine, neuroketal, oxyblot (8 post-baseline measurements: Weeks 4*, 12*, 24*, 36, and 48)
- Changes from baseline in plasma mediators of inflammation: interleukin 1 beta (IL-1β), interleukin 6 (IL-6), interleukin 8 (IL-8), interleukin 10 (IL-10), tumor necrosis factor alpha (TNF-α), interleukin 12 (IL-12), interleukin 17 (IL-17), interleukin 23 (IL-23), transforming growth factor beta 2 (TGF-β2), IL-1β (Western Blot), IL-10 (Western Blot), TNF-α (Western Blot), nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB) (Western Blot) (8 post-baseline measurements: Weeks 4*, 12*, 24*, 36, and 48)
- Changes from baseline in plasma non-directed metabolome profile: chromatogram or profile, peak identification (2 post-baseline measurements: Weeks 12* and 24*)
- Changes from baseline in plasma biomarkers of functional capacity of albumin

(Electron Paramagnetic Resonance [EPR], Ischemia Modified Albumin [IMA], oxidative status, Mass Spectrophotometry [MS]) (8 post-baseline measurements: Weeks 4*, 12*, 24*, 36, and 48)

- Changes from baseline in CSF standard analysis (cell count, glucose, total protein and albumin levels) (2 post-baseline measurements: Weeks 12 and 25)
- Changes from baseline in CSF oxidative stress: glutamic semialdehyde (GSA), malondialdehyde-lysine, carboxymethyl lysine (CML), carboxyethyl lysine (CEL) (2 post-baseline measurements: Weeks 12 and 25)
- Changes from baseline in CSF mediators of inflammation: interleukin 1 beta, interleukin 6, interleukin 10, tumor necrosis factor alpha (2 post-baseline measurements: Weeks 12 and 25)
- Changes from baseline in CSF non-directed metabolome profile: chromatogram or profile, peak identification (2 post-baseline measurements: Weeks 12 and 25)

* On these visits, biomarkers determinations were performed before and after the PE procedure

Safety:

The following safety variables were assessed:

- Percentage of PE sessions associated with at least one treatment-emergent adverse event (TEAE) /adverse reaction (AR) during or within 72 hours after the completion of the product infusion
- Percentage of PE sessions associated with at least one TEAE, irrespective of causality with the procedure
- Vital signs recorded at each assessment visit, before, during, and after each PE session, and as deemed necessary by the investigator
- Clinical laboratory testing (coagulation, blood count, biochemistry, and/or serology)
- Changes in laboratory parameters and vital signs that were clinically significant in accordance with investigator criteria.

Statistical Methods:

Data was presented using summary statistics.

All categorical variables were summarized in frequency and percentage.

The continuous variables were reported by sample statistics: n (number of observations), number of missing data, mean, standard deviation (SD), 95% confidence intervals (95% CI), minimum, first quartile (Q1), median, third quartile (Q3), and maximum.

For both primary efficacy variables the ALSFRS-R score and the FVC, changes from baseline were assessed in 5 measurements on weeks 4 (V1), 12 (V2), 25 (V4), 36 (V5), and 48 (V6) and were summarized by visit. In addition, ALSFRS-R score values for individual questions and functional subdomains were summarized by visit. Finally differences between baseline and subsequent visits for the ALSFRS-R bulbar/fine and gross motor/respiratory subdomains and overall were analyzed via a student t-test in the cases where the assumptions of normality were met. If the distribution of data was not shown to be normal, the nonparametric Wilcoxon signed-rank test was applied.

Concerning secondary efficacy variables, changes from baseline in EMG profile (sensory nerve conductor study [NCS] and motor NCS), ALS-CBS, ALSA-Q40, and different biomarkers such as non-directed metabolome profile (plasma/CSF), functional capacity of albumin (plasma), oxidative stress (plasma/CSF), and mediators of inflammation (plasma/CSF) were summarized by visit.

In addition, changes in plasma biomarkers of mediators of inflammation and oxidative stress before and after selected PE procedures (V1, V2, and V3) and changes in the plasma non-directed metabolome profile before and after selected PE procedures (V2, and V3) were summarized and analyzed using the non-parametric Wilcoxon test for paired data.

SUMMARY - CONCLUSIONS

EFFICACY RESULTS:

In the Evaluable Population, the ALSFRS-R showed a median (Q1, Q3) change of -4.0 (-8.0, -3.0) (p-value = 0.0010) and -10.0 (-14.0, -7.0) (p-value <0.0001) at V4 and V6, respectively, showing a statistically significant change from baseline in both study periods. According to the European Medicines Agency (EMA) guideline, (i.e. decrease of 1 point per month) the expected decline at V4 and V6 would have been -6.0 and -12.0, respectively, but the median decline found in the study was -4.0 and -10.0, respectively. However, when ALSFRS-R overall values were then compared with the expected decline statistical significance was not found in the change from baseline to V4 (p-value= 0.4941), or V6 (p-value = 0.8859).

When ALSFRS-R was divided into subdomains (bulbar, fine and gross motor and respiratory items), a statistically significant change from baseline to V6 was observed in the fine and gross motor item and the bulbar response, changes in the respiratory item were not observed at any time points.

On the other hand, statistically significant decreases in FVC and predicted FVC as a measure of pulmonary function were found at both the V4 and V6. Median (Q1, Q3) change from baseline of FVC predicted value was -9.0 % (-23.0, -6.0) (p-value = 0.0068) and -23.0 % (-38.0, -9.0) (p-value = 0.0006) at V4 and V6, respectively.

Regarding secondary efficacy variables, the behavioral and symptomatic status of the subjects did not show any statistically significant changes measured by the ALS-CBS test. Likewise, a decline in cognitive function was not observed throughout the study as analyzed by the ALS-CBS test.

Concerning EMG recordings, when comparing the baseline with final visit, motor NCS latency and amplitude results of the right/left tibialis anterior, thenar and hypothenar eminence showed statistically significant decreases throughout the study.

Regarding Quality of life evaluations measured through the ALSA Q40 test, no statistically significant changes were observed in communication and emotional functioning scores. However, a statistically significant increase (indicating a decrease in quality of life) was observed in the physical mobility, independence and eating and drinking items.

Finally, biomarker analysis in plasma and CSF suggested that PE procedure was able to produce transient changes, but these changes were not maintained over time and returned to baseline levels prior to the subsequent visit and at the end of the treatment phase.

SAFETY RESULTS:

All 13 subjects (100%) received at least 1 PE during the study. A total of 330/351 (94.0%) PE sessions were performed during the study using a peripheral route. No non-TEAEs were observed throughout the study. Twelve (12) subjects (92.3%) presented at least 1 TEAE. The overall proportion of PE procedures temporarily associated with any TEAEs potentially related to study procedure was of 0.9%.

The most common SOC were infections and infestations (53.8%), followed by nervous

system disorders (46.2%), gastrointestinal disorders (30.8%) and psychiatric disorders (30.8%). Two (2) subjects (15.4%) experienced at least one severe TEAE, including 3 TEAEs (pneumonia [7.7%, n=1], respiratory failure [7.7%, n=1] and mechanical ventilation [7.7%, n=1]).

One (1) subject (7.7%) had an Albutein potentially related TEAE (dizziness) and 1 subject (7.7%) a study procedure potentially related TEAE (headache). In addition, 1 subject (7.7%) had 2 TEAEs which were both product and procedure potentially related (presyncope and diarrhea). There were no more TEAEs potentially related to Albutein or to the procedure.

Three (3) subjects (23.1%) experienced a least 1 serious TEAE; pneumonia (n=2), pneumonia aspiration (n=1) and respiratory failure (n=1). However, none of these serious TEAEs were Albutein and/or study procedure related. Two (2) of these serious TEAE led to early withdrawal, in particular 1 subject (7.7%) suffered a pneumonia that led to treatment discontinuation and 1 subject (7.7%) died in the study due a respiratory failure.

Regarding clinical laboratory evaluations, 59 out 360 (16.4%) hematological determinations, 203 out of 657 (30.9%) biochemical measurements, and 15 out 95 (15.8%) coagulation results were considered as abnormal, respectively, but none of these abnormalities were considered clinically significant.

No clinically significant abnormalities in vital signs were reported. However, there were a total of 3 (0.37%) clinically significant and abnormal physical exams from available determinations were reported, all not related. In particular, 1 subject presented a common cold affecting eyes/nose body; 1 subject had respiratory difficulties in relation to ALS progression, and 1 subject experienced pneumonia, which was reported as a serious TEAE.

CONCLUSION:

Plasma Exchange (PE) with Albutein 5% treatment in ALS subjects showed for both co-primary variables (ALSFRS-R and FVC) a statistically significant progressive decrease throughout the study compared with baseline values, proving a general increase in the functional impairment. Individually, 7 out 13 subjects (53.8%) displayed a slower decline rate than the expected ALSFRS-R functional decline of about 1 point per month in untreated subjects on the ALSFRS-R score. The expected decline at V4 and the V6 would have been -6.0 and -12.0, respectively, but the median decline found in the study was -4.0 and -10.0, respectively. This difference from expected ALS natural progression was found not to be statistically significant.

In addition, when ALSFRS-R was divided into subdomains, a statistically significant change from baseline to V6 was observed in the fine and gross motor item and the bulbar response, but changes in the respiratory item were not observed at any time points, suggesting maintenance of the respiratory dysfunction level. At the same time, only 3 out of 11 subjects (27.3%) at V4 and 5 out of 11 subjects (45.5%) at V6 presented FVC values below 50%, which is indicative of poor pulmonary function, supporting the idea that respiratory dysfunction was not prominent among the studied ALS subjects.

Regarding secondary endpoints, results from EMG recordings and the ALSA Q40 scale supported the observation that subjects worsened compared with baseline measurements. On the other hand, the ALS-CBS test showed that cognitive function was maintained stable over time. Finally, biomarker analysis in plasma and CSF suggested that PE procedure was able to produce transient changes, but these changes were not maintained over time and returned to baseline levels prior to the subsequent visit and at the end of the

treatment phase.

From a safety perspective, PE procedures were shown to be safe and well-tolerated. The overall proportion of PE procedures temporarily associated with TEAEs potentially related to the study procedure was of 0.9%. Only 1 subject presented a TEAE (dizziness) potentially related to the study drug, 1 subject displayed a procedure potentially related TEAE (headache) and 1 subject presented 2 TEAEs which were both drug and procedure potentially related (presyncope and diarrhea). None of the TEAEs considered as serious were study drug and/or procedure related. Three (3) subjects experienced a serious TEAE (pneumonia, pneumonia aspiration and respiratory failure). One (1) subject suffered from a serious TEAE (pneumonia) that led to treatment discontinuation and 1 subject died during the study from respiratory failure as a consequence of ALS disease deterioration.

No clinical significant abnormalities were reported from vital signs and clinical laboratory evaluations (hematology, coagulation and biochemistry). Three (3) subjects experienced a clinically significant and abnormal finding, all not related during a physical examination (common cold, respiratory difficulties in relation to ALS progression, and pneumonia).

In conclusion, PE procedures with 5% Albutein show an adequate safety profile related in the treatment of ALS subjects. Regarding efficacy, ALS subjects experienced a statistically significant decline in disease progression throughout the study. However, individually and according to Investigator's judgement, a subgroup of subjects displayed a better than expected decline rate of ALSFRS-R, but given the limited sample size, this effect was not reflected in the group analysis.

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