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2 SYNOPSIS

Name of Sponsor / Company: Instituto Grifols, S.A.	
Name of Finished Product: Not applicable	
Name of Active Ingredient: Not applicable	
Title of Study: Pathogenesis of Acute on Chronic Liver Failure (ACLF) and Mechanisms of Action of Plasma Exchange with Human Serum Albumin 5% (PE-A 5%) in Decompensated Cirrhotic Subjects with Systemic Inflammation and ACLF	
Investigators: There were 11 sites with at least one subject enrolled. Refer to Appendix 16.1.4 for a list of participating investigators.	
Study Center(s): Refer to Appendix 16.1.4 for a list of participating study centers.	
Publication (reference): None.	
Studied Period (years): Date of first enrollment: 06 February 2020 Date of last completed: 27 March 2023	**Phase of Development**: Translational research study

Objectives: The objectives of this study were:

- To investigate the pathogenesis of systemic inflammation, organ failure (OF), and ACLF in cirrhosis.
- To explore mechanisms of action of PE-A 5% in subjects with ACLF.

Methodology: The ALADDIN study was a translational research study that was planned to analyze selected biological samples collected from subjects enrolled in the APACHE Clinical Trial (IG1407 study, a phase III, multicenter, randomized, controlled, parallel-group and open-label trial that evaluates the effects of PE-A 5% on short-term survival in subjects with ACLF at high risk of hospital mortality) and additional blood samples collected from newly recruited subjects with cirrhosis but without ACLF who served as the control group within this study.

A maximum of 250 subjects were to be enrolled in this study across 3 study groups, of which 150 were to be subjects with ACLF who had been enrolled and randomized in the APACHE study (IG1407). Group 1 was to be comprised of a maximum of 75 subjects with ACLF enrolled in the APACHE study who were randomized to the standard medical treatment (SMT) plus PE-A 5% (SMT+PE-A 5%) treatment group. Group 2 was to be comprised of a maximum of 75 subjects with ACLF enrolled in the APACHE study who were randomized to the SMT group. Group 3 was to include a maximum of 100 control subjects with cirrhosis without ACLF specifically recruited for the ALADDIN study.

Subjects enrolled in Groups 1 and 2 participated in both the APACHE study and the ALADDIN study. Blood samples from the subjects in Group 1 and Group 2 enrolled in the APACHE study were to be obtained on Day 1 of the ALADDIN study for analyzing all study variables. Follow-up determinations for kinome, inflammasome, transcriptome, pathogen associated molecular patterns (PAMPs), damage associated molecular patterns

(DAMPs), and systemic inflammation, microparticle (MP) identification, albumin functional capacity and coagulation were to be repeated on Day 6.

In addition, for subjects in Group 1 only, samples from plasma removed during PE-A 5% during the APACHE study were to be collected. On Day 1 and Day 6, plasma removed during the first 15 minutes of the PE-A 5% sessions in the APACHE study were to be obtained for MP identification. To assess the effect of PE-A 5% on albumin functional capacity, 2 additional samples were to be obtained from every PE-A 5% session in the APACHE study: 1 sample during the first 15 minutes and 1 sample from the last 15 minutes of the PE-A 5% session.

Blood samples from the subjects in Group 3 were to be collected at screening for confirming inclusion criteria, and at baseline for analyzing all the study variables. In these subjects, follow-up samples were not collected. If the amount of blood collected was not enough for analysis of all study variables proposed, analyses were to be prioritized as follows: cell isolation (kinome and inflammasome), transcriptome, PAMPs and DAMPs and systemic inflammation, MP identification, albumin functional capacity and coagulation. Kinome and inflammasome analyses in polymorphonuclear leukocytes (PMNs) were only to be performed in a subset of subjects enrolled at study centers with the capability for performing these specific analyses.

At the end of the study, after all planned analyses had been performed; any remaining samples were to be retained for future analysis related to ACLF pathogenesis and mechanisms of action of PE-A 5% in subjects with ACLF. However, the study was terminated early based on Sponsor's decision and available samples were analyzed only for the inflammasome and systemic inflammation study variables. All remaining blood samples for ALADDIN had been deposited and stored in a biomarker repository for potential later analysis within the scope of this study.

Number of subjects (planned and analyzed): A maximum of 250 subjects were to be enrolled in this study. However, a total of 147 subjects (22 subjects in Group 1, 25 subjects in Group 2, and 100 subjects in Group 3) were enrolled due to early termination of the study.

Diagnosis and Main Criteria for Inclusion: Subjects in Group 1 and Group 2 who met the APACHE inclusion and exclusion criteria and were enrolled and randomized to a study group in the APACHE study must met all the following inclusion criteria to be eligible for participation in the ALADDIN study:

1. Be enrolled at European study centers of the APACHE study which were also participating in the ALADDIN study.
2. Had signed the informed consent form (ICF) to participate in the ALADDIN study.

Subjects enrolled in Group 3 must have met all the following inclusion criteria to be eligible for participation in this study:

1. Male or female cirrhotic subject between 18 and 79 years of age.
2. Hospitalized for acute decompensation (AD) of cirrhosis (ascites, hepatic encephalopathy [HE], gastrointestinal hemorrhage and/or bacterial infections).

Bacterial infections were considered an AD of cirrhosis only in subjects with prior history of decompensated cirrhosis.

3. Willing and able to provide written informed consent or had an authorized representative able to provide written informed consent on behalf of the subject in accordance with local law and institutional policy.

Exclusion Criteria:

Subjects in Group 3 who met any of the following exclusion criteria were not eligible for participation in the study:

1v3. Subjects with ACLF (any grade) or had resolved ACLF in the last 7 days prior to screening.

2v3. Subjects with septic shock who required use of norepinephrine (>0.3 mcg/kg/min) or needed a second vasopressor (including terlipressin).

3v3. Subjects with active bacterial or fungal infection, who received less than 24 hours of appropriate antibiotic treatment.

4. Subjects with active or recent bleeding (unless controlled for >48 hours).

5. Subjects with chronic renal failure and were receiving hemodialysis.

6. Evidence of current locally advanced or metastatic malignancy (subjects with hepatocellular carcinoma within the Milan criteria [1 nodule ≤5 cm or 3 nodules ≤3 cm], non-melanocytic skin cancer, and controlled breast or prostate cancer, can be included).

7. Subjects with severe chronic heart failure (New York Heart Association class III or IV).

8. Subjects with severe pulmonary disease (Global Obstructive Lung Disease stage III or IV).

9v3. Subjects with HE stage 1 or higher according to West Haven criteria.

10. Subjects with severe critical illness myopathy as defined clinically.

11. Subjects with a known infection with human immunodeficiency virus (HIV) or clinical signs and symptoms consistent with current HIV infection.

12. Females who were pregnant.

13. Subjects with previous liver transplantation.

14v3. Subjects receiving anti-platelet or anti-coagulant therapy (low molecular weight heparin for deep vein thrombosis prophylaxis was allowed).

15. Participation in another clinical study within at least 30 days prior to screening.

16v3. Subjects with active drug addiction (exceptions: active alcoholism or marijuana use).

17. Subjects with a do-not-resuscitate order.

18. Subjects who, in the opinion of the investigator, had compliance problems with the protocol and the procedures of the protocol.

19. Subjects with current infection of COVID-19, those who were less than 14 days post recovery or those who had clinical signs and symptoms consistent with COVID-19 infection.

Investigational Product, Dose and Mode of Administration, Batch Number: No investigational product was administered during the ALADDIN study.

Duration of Treatment: Subjects in Group 1 were planned to participate in the ALADDIN study for up to 16 days depending on the number of PE-A 5% sessions completed during the APACHE study. For subjects in Group 2, the planned duration of study participation was 6 days. For subjects in Group 3, the duration of study participation was to be up to 2 days depending on when screening occurred.

Reference Therapy, Dose and Mode of Administration, Batch Number: No reference therapy was administered during the ALADDIN study.

Criteria for Evaluation:

Study Variables:
The following variables were planned to be studied in this study:

- Kinome in isolated PBMCs and PMNs.
- Inflammasome: nucleotide-binding oligomerization domain-lined receptors family pyrin domain containing 3, the adaptor molecule apoptosis-associated speck-like protein containing a C-terminal caspase recruitment domain, inactive pro-caspase-1, cysteine protease caspase-1 and pro-IL-1β in the active and secreted forms in isolated PBMCs and PMNs.
- Gene expression (transcriptome) in whole blood to identify which genes modify its expression when ACLF occurs or as a response of the treatment.
- PAMPs and DAMPs: Toll-like receptor 2, 4, and 9 agonists, and high-mobility group protein B1, respectively.
- Systemic inflammation: cytokines (EGF, G-CSF, GM-CSF, IFN-α2, IFN-γ, IL-1α, IL-1β, IL-1ra, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12 (p40), IL-12 (p70), IL-13, IL-15, IL-17A, IP-10, MCP-1, MIP-1α, MIP-1β, TNF-α, TNF-β, VEGF, Eotaxin/CCL11).
- Analysis of MPs: subtypes identification.
- Albumin functional capacity: albumin binding capacity, fatty acid binding capacity, antioxidant capacity and post-translational modifications.
- Coagulation: thrombin generation assay, thrombin generation on endothelial cells and coagulation parameters.
- Whole blood genome (genotyping) to identify gene predictors of ACLF and response to treatment.
- Bacterial DNA.

Safety:
Information related to assessment of safety of the subjects in Group 1 and Group 2 enrolled in the APACHE study was detailed in the APACHE study (IG1407) protocol. Adverse events occurring in subjects in Groups 1 and 2 were collected in the APACHE (IG1407) electronic case report forms (eCRFs) and subject's source documents for the APACHE study.

For subjects in Group 3, assessment of safety included collection and assessment of adverse events (AEs). Adverse events occurring at any time between signing of the subject's ICF and the last day of the subject's participation in the clinical trial were reported and recorded on the appropriate subject's eCRF entry and subject's source documents.

Statistical Methods:

Available data were included in summary statistics and no substitutions or imputations of missing values were performed. Categorical variables were summarized using counts (n) and percentages (%) and were presented in the form n (%). Continuous variables were summarized using mean, standard deviation (SD), first quartile (Q1), third quartile (Q3), minimum, maximum, median, and number of patients. All listings were sorted in order of treatment, subject, parameter (when applicable), and time of assessment (e.g., visit, time, and/or event).

Due to the early termination of the ALADDIN study, there was a change in the study variables to be assayed, only the inflammasome and systemic inflammation (cytokines) study variables were analyzed. Data collected in ALADDIN electronic data capture (EDC) database and the aforementioned study variables were analyzed and presented in the ALADDIN clinical study report (CSR).

Descriptive statistics of biomarker variables was presented by group and visit days, for all recorded results and change from baseline. The change in the inflammasome and cytokine in Group 1 and 2 from baseline (Day 1) to Day 6 was calculated as: Day 6 – Day 1. Sensitivity analysis was performed, which excluded subjects who enrolled the study, but failed to meet all inclusion or exclusion criteria. Due to early termination, not enough of subjects were enrolled, hence, no inferential analysis was conducted.

Determination of Sample Size

There was no formal sample size calculation. A maximum of 75 subjects each in Group 1 (SMT+PE-A 5%) and Group 2 (SMT), and a maximum of 100 subjects in Group 3 (non-ACLF control group) were planned to be enrolled in this study. However, due to early termination of the study, a total of 147 subjects (22 subjects in Group 1, 25 subjects in Group 2, and 100 subjects in Group 3) were enrolled.

SUMMARY - CONCLUSIONS

This study (ALADDIN) was terminated early based on Sponsor's decision concerning de-emphasis of sideline translational investigations (non-safety related decision). The APACHE trial was ongoing at the time of early termination of ALADDIN study. As a

result, APACHE EDC was not merged with ALADDIN EDC, hence, only information collected for Groups 1 and 2 under ALADDIN protocol have been summarized in the report along with Group 3 data.

EFFICACY RESULTS:
No efficacy analyses were performed in this study.

BIOMARKER RESULTS:
Due to early termination of this study, only a subset of the originally planned study variables (inflammasome and systemic inflammation [cytokines]) were analyzed and presented in the ALADDIN CSR. Plasma biomarkers were evaluated at Day 1 and Day 6 for Group 1 and Group 2, and only at Day 1 for Group 3.

Due to study early termination, only descriptive analyses of cytokines and inflammasome variables were performed. The absolute percent differences in the median values between groups at Day 1 were noted when the differences were >50% (arbitrarily assigned). The change in the cytokine and inflammasome values in Group 1 and Group 2 from Day 1 to Day 6 were reviewed for any increases or decreases in values over- time.

Typically, median values for cytokines at Day 1 were lowest in Group 3 (subjects with cirrhosis without ACLF; 17/21 cytokines) and highest in Group 2 (subjects with ACLF randomized to the SMT; 13/21 cytokines). Of the 8 cytokines in which median values were not highest in Group 2, 6 were highest in Group 1 (subjects with ACLF randomized to the SMT+PE-A 5%), indicating a possible trend for subjects with ACLF (Group 1 and Group 2) to have more similar cytokine levels that may be higher than subjects without ACLF.

For Group 2 compared to Group 3, 11/21 cytokines had an absolute difference in the medians >50% at Day 1, while in Group 1 compared to Group 3, 7/21 cytokines had an absolute difference in the medians >50% at Day 1. In Group 2 compared to Group 1 only 4/21 cytokines had an absolute difference in the medians >50% at Day 1. Based on these results, subjects with ACLF (Group 1 and Group 2) tended to have more similar cytokine levels than subjects with cirrhosis without ACLF (Group 3).

Cytokine median change (Day 6 - Day 1) values tended to show a reduction in Day 6 values compared to Day 1 values in Group 1 (subjects with ACLF randomized to SMT+PE 5%:18/21 cytokines) while cytokine median change values tended to increase in Day 6 values compared to Day 1 values in Group 2 (subjects with ACLF randomized to SMT: 16/21 cytokines) with the exception of some ILs. Based on the results in this small sample, it may be possible that PE-A 5% has an effect on some cytokine levels which tended to decrease.

In contrast to cytokines, inflammasome values at Day 1 tended to be highest in Group 3 (subjects with cirrhosis without ACLF; 9/14 inflammasomes) and lowest in Group 1 (subjects with ACLF randomized to SMT+PE-A 5%; 8/14 inflammasomes). Typically, median values for inflammasomes at Day 1 were comparable in Group 1 (lowest median

value in 8/14 inflammasomes) and Group 2 (lowest median value in 4/14 inflammasomes), indicating a possible trend for inflammasome levels to be lower in subjects with ACLF.

In general, inflammasome median change (Day 6-Day 1) values tended to show an increase in Day 6 values compared to Day 1 values in Group 1 and a decrease or no change in Group 2.

While no definitive clinical or statistical conclusions can be made based on the small sample sizes and variability of results, these results may warrant future investigation of cytokine and inflammasome biomarkers in these populations of patients.

SAFETY RESULTS:

No adverse events were reported in Group 3 subjects and therefore no safety concerns were identified. Group 1 and 2 safety data were reported as part of APACHE study and will be discussed in the APACHE study CSR.

CONCLUSION:

This study (ALADDIN) was terminated early based on Sponsor's decision concerning de-emphasis of sideline translational investigations (non-safety related decision). Given the limitations of the data and early termination, there was no conclusion drawn from the efficacy results. Also, no safety concerns were observed during this study (Group 3).