Research Article
Cirrhosis and Liver Failure

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Randomized, controlled clinical trial of the DIALIVE liver dialysis device versus standard of care in patients with acute-on-chronic liver failure

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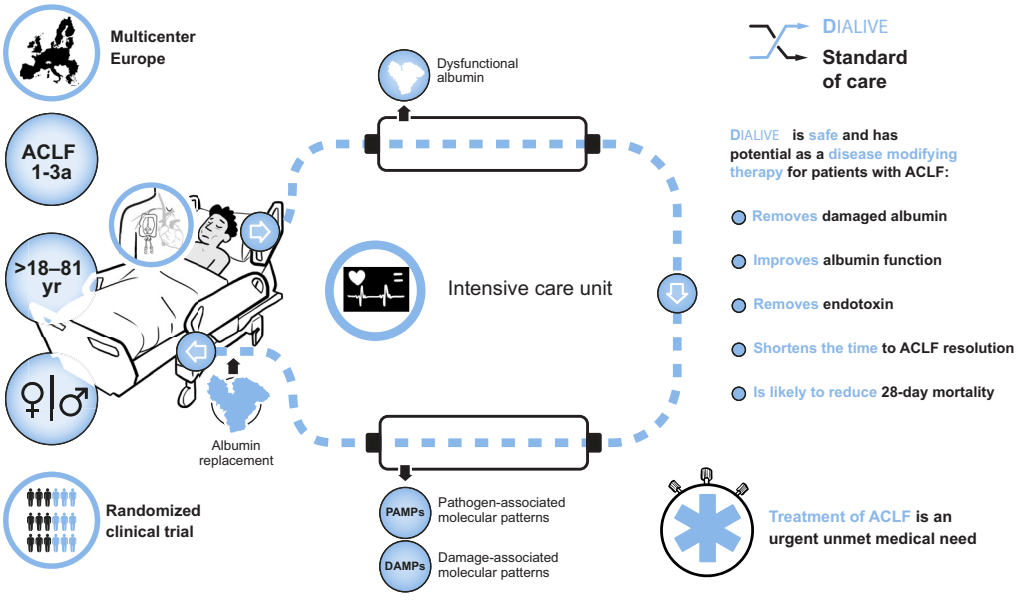
Graphical abstract



Highlights

- In a first-in-man, randomized-controlled trial of DIALIVE *vs.* standard of care, the primary endpoint of safety was met.
- DIALIVE achieved acceptable performance characteristics for albumin exchange and reduction in endotoxin.
- DIALIVE significantly reduced time to resolution of ACLF and improved prognostic scores compared with standard of care.
- DIALIVE had a significantly greater impact on the pathophysiologically relevant biomarkers associated with ACLF.

Impact and implications

This is the first-in-man clinical trial which tested DIALIVE, a novel liver dialysis device for the treatment of cirrhosis and acute-on-chronic liver failure, a condition associated with severe inflammation, organ failures and a high risk of death. The study met the primary endpoint, confirming the safety of the DIALIVE system. Additionally, DIALIVE reduced inflammation and improved clinical parameters. However, it did not reduce mortality in this small study and further larger clinical trials are required to re-confirm its safety and to evaluate efficacy.This is the first-in-man clinical trial which tested DIALIVE, a novel liver dialysis device for the treatment of cirrhosis and acute-on-chronic liver failure, a condition associated with severe inflammation, organ failures and a high risk of death. The study met the primary endpoint, confirming the safety of the DIALIVE system. Additionally, DIALIVE reduced inflammation and improved clinical parameters. However, it did not reduce mortality in this small study and further larger clinical trials are required to re-confirm its safety and to evaluate efficacy.

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Randomized, controlled clinical trial of the DIALIVE liver dialysis device versus standard of care in patients with acute-on- chronic liver failure

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Background & Aims: Acute-on-chronic liver failure (ACLF) is characterized by severe systemic inflammation, multi-organ failure and high mortality rates. Its treatment is an urgent unmet need. DIALIVE is a novel liver dialysis device that aims to exchange dysfunctional albumin and remove damage- and pathogen-associated molecular patterns. This first-in-man randomized-controlled trial was performed with the primary aim of assessing the safety of DIALIVE in patients with ACLF, with secondary aims of evaluating its clinical effects, device performance and effect on pathophysiologically relevant biomarkers.
Methods: Thirty-two patients with alcohol-related ACLF were included. Patients were treated with DIALIVE for up to 5 days and end points were assessed at Day 10. Safety was assessed in all patients (n = 32). The secondary aims were assessed in a pre-specified subgroup that had at least three treatment sessions with DIALIVE (n = 30).
Results: There were no significant differences in 28-day mortality or occurrence of serious adverse events between the groups. Significant reduction in the severity of endotoxemia and improvement in albumin function was observed in the DIALIVE group, which translated into a significant reduction in the CLIF-C (Chronic Liver Failure consortium) organ failure ($p = 0.018$) and CLIF-C ACLF scores ($p = 0.042$) at Day 10. Time to resolution of ACLF was significantly faster in DIALIVE group ($p = 0.036$). Biomarkers of systemic inflammation such as IL-8 ($p = 0.006$), cell death [cytokeratin-18: M30 ($p = 0.005$) and M65 ($p = 0.029$)], endothelial function [asymmetric dimethylarginine ($p = 0.002$)] and, ligands for Toll-like receptor 4 ($p = 0.030$) and inflammasome ($p = 0.002$) improved significantly in the DIALIVE group.
Conclusions: These data indicate that DIALIVE appears to be safe and impacts positively on prognostic scores and pathophysiologically relevant biomarkers in patients with ACLF. Larger, adequately powered studies are warranted to further confirm its safety and efficacy.
Clinical trial number: NCT03065699.

Introduction

Acute-on-chronic liver failure (ACLF) occurs in hospitalized patients with cirrhosis who present with acute decompensation with a liver-related complication.[1] It is characterized clinically by multiorgan failure and a high risk of short-term mortality and, pathophysiologically, by the presence of systemic inflammation.[2] Mechanistically, severe albumin dysfunction and accumulation of damage- and pathogen-associated molecular patterns (DAMPs and PAMPs) are thought to contribute significantly to the systemic inflammation observed in ACLF.[3–5]

The European Association for the Study of Liver diseases – Chronic Liver Failure (EASL-CLIF) Consortium criteria for the diagnosis of ACLF and prognosis of these patients have been well-validated.[1] The CLIF-Consortium (CLIF-C) ACLF score, which is a composite score derived from CLIF-C organ failure

Keywords: acute-on-chronic liver failure; DIALIVE; extracorporeal liver dialysis; Albumin.
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(CLIF-OF) score, patient age and white cell count has been validated as a more accurate score than the conventionally used scoring systems in defining the prognosis of patients with ACLF.[6] Depending upon the severity of ACLF, resolution occurs in about 20-55% of patients with the current standard of care (SOC).[6] Once recovered, the survival rates approach those without ACLF. Therefore, the short-term goal of therapy is to increase the proportion of patients that resolve ACLF and reduce the time to resolution.[7]

Several extracorporeal liver assist devices have been tested but they have not been shown to improve survival of patients with ACLF.[8–10] Apart from liver transplantation, no treatment has been shown to reduce mortality in these patients and its treatment is thus an important unmet need.[11] Currently, the management of ACLF involves treatment of the specific complications and multiorgan supportive care.[1]

DIALIVE is an extracorporeal liver dialysis device that has been built to specifically address the pathophysiological derangements responsible for the development of ACLF.[12] DIALIVE incorporates a renal dialysis machine (Prismaflex, Baxter) and uses a dual filtration system connected in series. The first filter is comprised of a membrane that allows ultrafiltration of albumin and cytokines (Septex, Baxter, USA) and the second filter adsorbs PAMPs, such as endotoxins, and DAMPs, such as genomic DNA (Oxiris, Baxter, USA). The removed albumin is replaced in similar quantities with bottled, 20% albumin. The rationale underlying DIALIVE is the following. First, the circulating albumin in ACLF is not only dysfunctional but can itself induce an inflammatory response.[13,14] Second, systemic inflammation is the result of the accumulation of DAMPs and PAMPs, which lead to organ immunopathology and increased risk of infections.[15,16] Therefore, DIALIVE aims to replace albumin and remove DAMPs and PAMPs. In large animal models of liver failure, DIALIVE was shown to be safe, demonstrated evidence of device performance and reduced short-term mortality, thereby providing the rationale to initiate clinical trials.[12]

This multicenter, randomized-controlled trial of DIALIVE *vs*. SOC was designed to test its safety and verify the hypothesis that DIALIVE will significantly improve the prognostic scores of patients with alcohol-related cirrhosis and ACLF by impacting on the pathophysiological mechanisms of the condition and resolving organ failure.

Patients and methods

Patient eligibility

The study (NCT03065699) was approved by the relevant Institutional Review Boards of the participating sites and was conducted according to the protocol, the ISO14155, the ethical principles originating from the Declaration of Helsinki, and consistent with ICH Guidelines. Patients were required to supply written informed consent prior to participating. The study protocol(s) are presented as Appendix 1. During the study, two major and a few minor amendments were made to the protocol. The first major amendment included incorporation of recommendations of the Data Safety Monitoring Board (DSMB) to make DIALIVE therapy safer with detailed treatments to be carried out within an intensive care unit (ICU) environment with frequent monitoring, immediate recognition and treatment of hypotensive episodes, volume pre-loading in clinically hypovolemic patients and albumin replacement at the same time as removal (Appendix 2). In both groups, ICU admission was mandated in case of the requirement for circulatory, renal or respiratory organ support or, the need for airway protection in the case of severe hepatic encephalopathy. The second major amendment was expansion of inclusion criteria to allow patients with ACLF grade 3 (maximum 3 OFs; grade 3a) to be enrolled and for serum bilirubin above 20 mg/dl and serum creatinine above 1.5 mg/dl to be ACLF-defining diagnoses (Appendix 1).

This was a multicenter, European, randomized-controlled, open-label study to generate data from the evaluation of safety and performance of a novel liver dialysis device, DIALIVE, in patients with ACLF *vs.* SOC. Cirrhosis was defined by clinical, biochemical, or histological evidence.

Patients had to be 18 years or over with ACLF Grades 1-3a. Inclusion and exclusion criteria are described in the protocol. They were assigned to five cohorts. Each cohort consisted of six patients (three DIALIVE: three SOC). Dropouts in any of the cohorts were replaced by new patients to ensure accrual of six evaluable patients per cohort. Data from each cohort were reviewed for safety by the DSMB before proceeding to the next cohort.

Study design and treatment

Patients were recruited from in-patient wards. Randomization was performed electronically with an interactive wireless randomization system. Patients were randomized and then followed for a maximum treatment window period of 10 days (DIALIVE arm), which is the time used for evaluation of device performance, clinical efficacy, and exploratory endpoints. A minimum of three DIALIVE sessions of 8-12 h each were needed for the patient to be evaluable for efficacy assessment. Set up time for each DIALIVE session was about 45 min. Operational characteristics and set-up of DIALIVE are summarized in Fig. S1.

All patients were followed for 28 days, and those from Cohorts 4 (2 patients) and 5 (6 patients) were followed for 90 days (Fig. S2). The main time points for data and sample collection were at baseline, Day 5, and Day 10.

Removal of patients from therapy or assessment

The participant (or their legal representative) was allowed to voluntarily withdraw from the study at any time for any reason. The investigator also had the right to withdraw a patient at any time due to failure to follow the clinical investigation plan, or for administrative, safety or other reason(s). Stopping rules (for the study and for the DIALIVE treatment) are described in the protocol (Appendix 1, 2).

Endpoints and assessments

Primary endpoint

The goal was to evaluate the percentage of patients who experienced at least one serious adverse event (SAE) between study Day 1 (first day of treatment) and Day 10, especially the incidence rate of SAEs between the study groups occurring in this period, as well as to determine the percentage of patients who discontinued DIALIVE due to a serious adverse device event (SADE) between Day 1 and Day 10 (applicable to DIALIVE only).

Secondary endpoints

To evaluate the performance of the DIALIVE device as measured by change in plasma endotoxin level (endotoxin

activity and concentration), albumin function, 28-day mortality; change in individual organ function, in CLIF-OFs, ACLF grade and CLIF-C ACLF score; ICU and hospital stay (Appendix 1).

Exploratory endpoints

Exploratory endpoints included the effects of DIALIVE compared to SOC on organ function and pathophysiological markers of inflammation (Appendix 1).

Biobanking and analysis of bio samples

The bio samples were centrally bio banked and the analysis was performed by independent groups blinded to the treatment applied. See Appendix 3 for biomarker measurement methods and for biobanking details.

Statistical analysis

The data analysis was performed by an independent group (IDIBAPS) under the direction of the Data Management Centre (EF-CLIF), for regulatory purposes by an independent group (PL, CA) (Fig. S3) and re-checked by another independent statistician (JC, Incliva, Spain). No specific hypothesis was to be statistically assessed in this study. As this was a first-in-man study, it was not powered to detect any pre-planned safety or efficacy differences. All statistical testing was therefore *post hoc* and exploratory.

The safety population (safety set) was defined as the subset of randomized patients who received at least one session of treatment (in the DIALIVE arm). The modified safety population (modified safety set) only included the evaluable patients to estimate the efficacy endpoints, including biomarkers. Values are reported as mean and standard deviation if the variable is quantitative and as frequencies and percentages otherwise.

Mixed models for repeated measurements (MMRM) analysis was performed to evaluate the statistically significant differences between and within groups (SOC and DIALIVE) for biomarkers and efficacy endpoints at the main time-points (Day 5 and Day 10) in the modified safety set. Reported p values, effect sizes and 95% CIs of time effect and within treatment comparisons were obtained using MMRM analysis for absolute values adjusted by treatment, time, and interaction between time and treatment. Overall treatment effect was calculated using absolute differences to baseline adjusted by time, treatment and interaction between both. Individual organ scores were evaluated using cumulative link mixed models with Laplace approximation adjusted by treatment, time and interaction between time and treatment. The effect of resolution of ACLF was studied using a two-way ANOVA for absolute values adjusted by resolution, time and interaction between both. All p value calculations are two-sided and no p value adjustment was performed except for the ANOVA model where the Tukey method was used to control family-wise error rate. Kaplan-Meier curves were constructed to compare time to resolution between groups and differences were assessed using log-rank statistics. SAS 9.4 was used for all MMRM, and R 4.0.1 was used for cumulative link mixed models and ANOVA (statistical analysis plan, Appendix 4).

Results

Study conduct and patient characteristics

A total of 32 patients with ACLF were included, DIALIVE (n = 17) *vs*. SOC (n = 15), in eight European hospitals in six countries, between July 2017 and January 2020.

Baseline patient characteristics are summarized in Table 1. Mean age was 49 years and approximately 75% of patients were male. All had clinical, radiological, or histological evidence of underlying alcohol-related cirrhosis. The precipitating event in all patients was alcohol-related hepatitis (NIAAA criteria[17]) with superimposed infection in four cases in each group. Six patients in each group were corticosteroid non-responders; the rest had contraindications to steroids or steroids were not considered according to local practice. Bacterial infection was controlled at the time of randomization. Two patients were replaced according to the study protocol due to early deaths. Therefore, a safety population was defined and referred to as DIALIVE-safety (32 patients) for all safety analyses and a modified safety population (DIALIVE-modified safety) (n = 30; Table S1) was used for efficacy assessments (Fig. S4; CONSORT diagram).

DIALIVE treatment and device deficiency

DIALIVE therapy was administered for a median of three sessions (range 1-5), with each session lasting 8-12 h, in the first 3 days (range 1-6). Two patients were treated for 1 day, 11 for 3 days, one for 4 days and three for 5 days. User errors occurred in three patients and filter clotting occurred in five patients. The latter resolved with filter replacement (details in Table S2).

Safety

Over the specified study period, two patients in the DIALIVE group died after the first session and did not complete three treatments. The first death was caused by hypotension due to possible sepsis and the second death resulted from a combination of sepsis, hypotension and disseminated intravascular coagulation. These early deaths were analyzed by the DSMB after the second patient death, which occurred in the second study cohort and recommendations for close safety monitoring were instituted, culminating in a protocol change (details of changes described in Appendix 2). Within 28 days, two other patients died in the DIALIVE group and three died in the SOC group (Box 1) (Appendix 5 provides narratives related to serious adverse events [SAEs] and deaths). All the deaths in the SOC group were thought to be liver-related whereas in the DIALIVE group, one death was consequent upon myocardial infarction and one due to progressive liver failure in a patient with ACLF grade 3 and a CLIF-ACLF score of 66 at the time of randomization. Between 28- and 90-days post-randomization, a further one SOC- and one DIALIVE-treated patient died.

Treatment-emergent AEs were reported in 76.5% of DIALIVE patients and 80% of SOC patients (Table 2), without significant differences between both groups (95% CI 0.35-0.29, p = 1.000). Regarding SAEs, 64.7% of patients in the DIALIVE group and 53.3% of patients in the SOC group experienced at least one SAE (Table 3; 95% CI 0.29-0.51,

Table 1. Baseline patient characteristics: safety set (n = 32).

Variable	DIALIVE (n = 17)	SOC (n = 15)
Demographics		
Age (years), mean (SD)	49 (12.3)	49.1 (10.2)
Male sex, n (%)	13 (76.5)	11 (73.3)
Race, n (%)		
Asian	0 (0)	1 (6.7)
Caucasian	13 (76.5)	13 (86.7)
Other	4 (23.5)	1 (6.7)
BMI (kg/m^2), mean (SD)	29.6 (5.7)	29.6 (6.1)
Medical history, n (%)		
At least one comorbidity	6 (35.3)	5 (33.3)
Chronic kidney disease	1 (5.9)	1 (6.7)
Cardiac disease	2 (11.8)	1 (6.7)
Pulmonary disease	3 (17.6)	4 (26.7)
Neurological disorder	1 (5.9)	0 (0)
Gastrointestinal disease	1 (5.9)	2 (13.3)
Liver disease, n (%)		
Alcohol-related cirrhosis	17 (100)	15 (100)
First decompensation	9 (52.9)	10 (66.7)
Cause of decompensation, n (%)		
Infection	4 (23.5)	4 (26.7)
Alcoholic hepatitis	17 (100)	15 (100)
Steroid administration	6 (35)	6 (40)
Laboratory values, mean (SD) or median (range)		
Albumin (g/L)	30.2 (6.8)	32.1 (6.4)
Sodium (mEq/L)	134.2 (6.1)	134.7 (4)
Ammonia (μmol/L)	62.2 (19.6)	63 (31.9)
Lactate (mmol/L)	1.5 (0.7)	1.6 (0.8)
Bicarbonate (mEq/L)	22.8 (4.7)	21.6 (4.1)
Hemoglobin (g/L)	95.3 (23.9)	84.9 (23.4)
White cell count (10^9/L)	14.2 (10.2)	11.7 (5.8)
Platelet count (10^9/L)	237 (40-312)	115 (35-274)
C-Reactive Protein (mg/L)	38.4 (21.7)	31.5 (25.2)
Scores		
Lille score, mean (SD)	0.5 (0.4)	0.5 (0.4)
MELD, mean (SD)	27.2 (7)	26.1 (7.3)
MELD Na, mean (SD)	28.6 (12.3)	26.6 (8.8)
Child-Pugh score, mean (SD)	11.6 (1.6)	10.9 (1.1)
CLIF-C OF, mean (SD)	10.3 (1.6)	9.7 (1.0)
CLIF-C ACLF score, mean (SD)	48.8 (8.4)	46.5 (5.5)
Liver score, n (%)		
1	0 (0)	1 (7)
2	2 (12)	0 (0)
3	15 (88)	14 (93)
Total bilirubin (mg/dl), mean (SD)	24.6 (13)	25.1 (11)
Kidney score, n (%)		
1	12 (71)	11 (73)
2	3 (18)	1 (7)
3	2 (12)	3 (20)
Creatinine (mg/dl), mean (SD)	1.5 (0.9)	1.5 (1.2)
Renal replacement therapy, n (%)	1 (6)	2 (13)
Brain score, n (%)		
1	2 (12)	7 (47)
2	14 (82)	8 (53)
3	1 (6)	0 (0)
Coagulation score, n (%)		
1	10 (59)	10 (67)
2	4 (24)	1 (7)
3	3 (18)	4 (27)
INR, mean (SD)	1.9 (0.6)	2.0 (0.8)
Circulation score, n (%)		
1	16 (94)	14 (93)
2	0 (0)	0 (0)
3	1 (6)	1 (7)
MAP (mmHg), mean (SD)	93 (15)	93 (10)
Use of vasopressors, n (%)	1 (6)	1 (7)
Lung score, n (%)		
1	14 (82)	13 (87)
2	2 (12)	2 (13)
3	1 (6)	0 (0)
SpO_2/FiO_2, mean (SD)	405 (113)	437 (47)
Mechanical ventilation, n (%)	1 (6)	0 (0)
ACLF grade, n (%)		
1	11 (65)	8 (53)
2	3 (18)	6 (40)
3	3 (18)	1 (7)

ACLF, acute-on-chronic liver failure; CLIF-C, Chronic Liver Failure Consortium; INR, international normalized ratio; MAP, mean arterial pressure; MELD, model for end-stage liver disease; OF, organ failure.

p = 0.769). Of these, 41.2% of DIALIVE patients were considered to have had a related SAE. The most common SAEs in the DIALIVE and SOC patients were hypotension (52.9% *vs.* 20%) and thrombocytopenia, anemia, or bleeding (47.1% *vs.* 26.7%) and bacterial infection (53.3% *vs.* 35.3%) respectively (Table 3; Appendix 5). The issue with hypotension was largely resolved following implementation of the DSMB guidance for patient management. However, two further transient episodes of hypotension were reported (Appendix 5). The first was associated with concomitant stopping of terlipressin and was corrected promptly with re-introduction. The second was in a patient with concomitant sepsis, which was corrected rapidly with fluids and inotropes. Need for antibiotics, inotropes and renal replacement therapy were similar between the groups (Table S3).

Device performance

Albumin ultrafiltration

Over a treatment period of 8 h, a mean of 41.1 (SD 11.7) g of albumin was lost in the dialysate on the first day of DIALIVE with no significant difference hour per hour during the 8 h of dialysis. Similar amounts of albumin were lost on each day of DIALIVE (Table S4).

Albumin function

No trends towards any differences in albumin concentrations were observed between groups at Day 5 (p = 0.529) or Day 10 (p = 0.792). Therefore, any changes observed in albumin function would likely be attributable to the intervention (Table S5). The main functional domains of albumin were tested. There was a significant increase in human mercaptalbumin (HMA) (p = 0.001 and p <0.001) and a reduction in both human non-mercapt albumin (HNA)-1 (p = 0.023 and p = 0.005) and HNA-2 (p = 0.002 and p = 0.017) at both Days 5 and 10 in the DIALIVE group compared with SOC, resulting in a significant increase in albumin redox status as reflected by an increase in the HMA/HNA ratio (0.491 [0.164; 0.817], p = 0.004 and 0.554 [0.227; 0.881], p = 0.002 at Days 5 and 10, respectively) (Fig. 1; Table S5). Electron parametric resonance spectroscopy was performed to evaluate the functional efficiency of the albumin binding sites. Two composite measures were analyzed: binding and detoxification efficiencies of albumin. There was a significant increase in the binding efficiency of albumin at Day 10 in the DIALIVE group

Box 1. Causes and timing of 28-day mortality (safety set).

DIALIVE (n = 4/17)

Day of death from randomization: 2
Severity at randomization: ACLF score of 60.5 and ACLF grade of 2
Cause: hypotension followed by multi-organ failure
Relationship to intervention: Investigator: unrelated; DSMB assessment: possibly related to therapy

Day of death from randomization: 3
Severity at randomization: ACLF score of 37.9 and ACLF grade of 2
Cause: hypotension, septic shock and DIC
Relationship to intervention: Investigator: Hypotension: related; Septic shock: probably related; DIC: possibly related; DSMB assessment: Hypotension: related; Septic shock: unrelated; DIC: related

Day of death from randomization: 19
Severity at randomization: ACLF score of 66 and ACLF grade of 3
Cause: multiorgan failure
Relationship to intervention: Investigator: unrelated; DSMB assessment: unrelated

Day of death from randomization: 9
Severity at randomization: ACLF score of 60.13 and ACLF grade of 2
Cause: myocardial infarction
Relationship to intervention: Investigator: unrelated; DSMB assessment: unrelated

Standard of care (n = 3/15)

Day of death from randomization: 9
Severity at randomization: ACLF score of 49.4 and ACLF grade of 2
Cause: sepsis followed by a terminal gastrointestinal bleed

Day of death from randomization: 21
Severity at randomization: ACLF score of 45.1 and ACLF grade of 2
Cause: acute kidney injury, worsening liver dysfunction and multiorgan failure

Day of death from randomization: 18
Severity at randomization: ACLF score of 45.83 and ACLF grade of 1
Cause: acute kidney injury, upper gastrointestinal bleed, cardiac arrest, worsening liver dysfunction and multiorgan failure

ACLF, acute-on-chronic liver failure; DIC, disseminated intravascular coagulation.

compared with the SOC (p = 0.016), while detoxification efficiency did not reach statistical significance (Fig. 1; Table S5). The function of the metal binding domain was measured as ischemia-modified albumin ratio, which was significantly reduced in the DIALIVE group compared with the SOC group at both Days 5 (p <0.001) and 10 (p = 0.009) (Fig. 1; Table S5).

Severity of endotoxemia

Three endotoxin measures were performed to evaluate the effect of DIALIVE on the severity of endotoxemia.

1. Endotoxin activity assay. Reliable measures (coefficient of variation <15%) were obtained in 21 patients (DIALIVE: 10; SOC: 11). There were trends towards reduction in the severity of endotoxemia in the DIALIVE group which was most marked at Day 5, but the differences were not statistically significant (-0.293 [-0.697; 0.111], p = 0.145). The patient-level pre-defined reduction goals were 40% as the target; a 20% reduction was considered acceptable. Target values were reached in 3 out of 10 patients (30%) on DIALIVE and 0 out of 11 (0%) on SOC at Day 5 (p = 0.090). Acceptable values were reached in 8 out of 10 patients (80%) on DIALIVE and 4 out of 11 patients (36.4%) on SOC (p = 0.081) at Day 5. By Day 10, the advantage observed for DIALIVE was not retained.
2. Limulus amebocyte lysate assay. No significant effect was found at Day 5 (p = 0.152), but there was a statistically significant advantage for DIALIVE at Day 10 (p = 0.001).
3. Lipoprotein binding protein did not show any significant differences (Fig. 4A; Table S5).

Clinical efficacy

The changes in laboratory parameters are shown in Table S6.

Changes in organ function and related prognostic scores

There were significant improvements in the liver (p <0.001), kidney (p <0.001), coagulation (p <0.001) and brain (p <0.001) sub scores of the CLIF-C OF score in both groups but the changes in each of these sub-scores were significantly greater in the DIALIVE group at Day 10 (Table S7; Fig. 2). Although the ACLF grades were not statistically different between groups, six (42.9%) patients on DIALIVE compared to four (26.7%) on SOC achieved ACLF resolution at Day 10 (p = 0.450). All patients in either group who resolved ACLF were on antibiotics, either initiated beforehand or at the time of randomization. The time to resolution of ACLF was significantly faster in patients on DIALIVE (log rank test, p = 0.036) (Fig. 2C). Length of stay in ICU was available for 24 of the 32 randomized patients. In these patients, the mean ICU length of stay was 6.6 ± 3.2 days for DIALIVE and 8.2 ± 2.6 days for the SOC group. There was no treatment effect overall on CLIF-C OF score (p = 0.260) or ACLF score (p = 0.134) but a significant decrease was observed in the DIALIVE group at Day 10 (differences between groups: -1.271 [-2.316; -0.226], p = 0.018 for CLIF-C OF score and -4.2 [-8.72; -0.176], p = 0.042 for ACLF score) (Fig. 2). No significant changes were observed for the MELD (model for end-stage liver disease) score (p = 0.256).

Pathophysiologic effects

To determine whether the clinical effects of DIALIVE were associated with changes in the known pathophysiological mechanisms underlying ACLF, several biomarkers were measured at similar time points to the clinical assessments.

Systemic inflammation: Fifteen markers of inflammation were measured (Table S6). For IL-8, there was an overall treatment effect (p = 0.008). There was a significantly larger reduction in IL-8 levels in the DIALIVE group at both Day 5 (-43.355 [-85.390; -1.320], p = 0.044) and Day 10 (-61.231 [-103.266; -19.196], p = 0.006). For TNF-α, there was no significant treatment effect (p = 0.094) (Fig. 3). There were trends to reduction that were consistent in the DIALIVE group but more variable in the SOC group. Statistically significant changes from baseline were observed in the DIALIVE group for IL-1β (p = 0.026 at Days 5 and 10), IL-18 (p = 0.021 at Day 5), CXCL1 (p = 0.010 at Day 10), CCL5/RANTES (p = 0.015 at Day 10). No statistically significant changes were observed for IL-6, IL-7, CX3CL1, sCD63, and CCL2/MCP1 in either group (Fig. 4A).

DAMPs: For the M30 component of cytokeratin-18 there was a significant treatment effect overall (p = 0.002), and a

Table 2. Overall summary of adverse events.

Parameter; n (%)	DIALIVE (n = 17)	SOC (n = 15)
Presence of AE (AE)	13 (76.5)	12 (80)
Presence of Related AE (RAE)	10 (58.8)	0 (0)
Presence of Serious AE (SAE)	11 (64.7)	8 (53.3)
Presence of Related Serious AE (R)	7 (41.2)	0 (0)
Presence of Device AE (ADE)	10 (58.8)	0 (0)
Presence of Serious Device AE (SADE)	7 (41.2)	0 (0)
Presence of Unexpected Serious Device AE (USADE)	3 (17.6)	0 (0)
Presence of Device Deficiency AE (DD)	2 (11.8)	0 (0)

AE, adverse event; SOC, standard of care.

significant reduction at Day 10 in the DIALIVE group ($p = 0.005$). Similarly, for the M65 component there was a significant treatment effect overall ($p = 0.028$) and a significant advantage for DIALIVE at Day 10 ($p = 0.029$). For receptor-interacting serine/threonine-protein kinase 3 (RIPK3) there was no treatment effect overall ($p = 0.094$) but there was a significant advantage for DIALIVE at Day 5 ($p = 0.030$) (Fig. 3).

Toll-like receptor 4 and inflammasome ligands: There was a significant treatment effect overall ($p = 0.003$) with significant reduction in the DIALIVE group at both Day 5 ($p = 0.005$) and Day 10 ($p = 0.030$) when the patient's plasma was incubated with a Toll-like 4 receptor (TLR4) reporter cell line. Similarly, there was a significant treatment effect ($p < 0.001$) when the patient's plasma was incubated with the IL-1β/IL-18 inflammasome cell line. There were significant advantages for DIALIVE at both Day 5 ($p < 0.001$) and Day 10 ($p = 0.002$) (Fig. 3).

Endothelial dysfunction: For asymmetric dimethylarginine (ADMA) there was a significant treatment effect overall ($p = 0.001$) with a significant reduction in the DIALIVE group at Day 10 ($p = 0.002$). For Factor VIII, there was a significant treatment effect overall ($p = 0.009$) with significantly greater reduction observed at Day 5 ($p = 0.002$) in the DIALIVE group. Although not statistically significant, there were trends towards reduction in E-Selectin, ICAM-1 and VCAM-1 that appeared more marked in the DIALIVE group (Fig. 4A).

Ammonia and symmetric dimethylarginine: Decreases from baseline were observed for ammonia in the DIALIVE group through Day 5, but this was not statistically significant (Table S6). Symmetric dimethylarginine is a stereo isomer of ADMA and a sensitive measure of renal function. There was a significant treatment effect overall ($p = 0.021$) with a significant advantage for DIALIVE at Day 5 ($p = 0.040$) (Table S6).

Clinical and pathophysiological factors associated with ACLF resolution

Resolution of ACLF in the DIALIVE group was associated with trends to improvements in most of the biomarkers measured with significant changes in coagulation factor VIII ($p = 0.032$), IL-18 ($p = 0.002$), M30 component of cytokeratin-18 ($p = 0.018$) and RIPK3 ($p = 0.031$). In the SOC group, there was an apparently paradoxical relationship with CCL5/Rantes ($p = 0.004$) and M65 component of cytokeratin-18 ($p = 0.029$) being associated with resolution of ACLF. A reduction in international normalized ratio was associated with resolution of ACLF ($p = 0.022$) (Fig. 4B).

To determine whether there were general factors associated with resolution of ACLF, both groups were combined (Fig. 4C). In general, the data suggested that a reduction in the pathophysiologic factors studied is associated with ACLF resolution in all major domains. The data showed that a reduction in coagulation factor VIII ($p = 0.018$), IL-7 ($p = 0.027$), IL-18 ($p = 0.030$), RIPK3 ($p = 0.034$) and international normalized ratio ($p = 0.011$), and an increase in CCL5/Rantes ($p = 0.003$), were associated with resolution of ACLF.

Discussion

This randomized clinical trial of DIALIVE *vs.* SOC in patients with ACLF Grades 1 to 3 provides data, indicating the safety of DIALIVE as evidenced by the similar proportion of patients experiencing serious and treatment emergent AEs in the DIALIVE and SOC groups. However, there were two early deaths in the DIALIVE arm. Following the two early deaths, changes in the guidance to patient and device management were provided in consultation with the DSMB after the recruitment of Cohort 2. The statistically significant effect of DIALIVE therapy on the severity of endotoxemia and albumin function suggests evidence of device performance. From the efficacy standpoint, the results suggest that treatment with DIALIVE results in a more rapid resolution of ACLF. Furthermore, this was associated with significant impact on the known pathophysiological mechanisms underlying ACLF development, such as markers of systemic inflammation, DAMPs and PAMPs, endothelial function and ligands of the TLR4 and inflammasome pathways.[1–5] However, given the relatively small sample size, these data must be interpreted cautiously.

This is the first study of an extracorporeal liver assist device in patients with ACLF using well-validated diagnostic and prognostic criteria. All patients included in this study had severe alcohol hepatitis that were either unresponsive, had contraindications or thought to be inappropriate for corticosteroid therapy according to local guidelines, and 27% also had a concomitant infection. A 28-day cumulative mortality of 21.8% at 28 days is in keeping with current literature.[1,2]

Apart from user errors and clotting of the filters, there were no significant technical issues with the application of DIALIVE. Patients with ACLF are known to have severely deranged coagulation with some patients having a pro-coagulant state.[17] Therefore, filter clotting is common in ACLF even in those having only renal replacement therapy.[18] The anticoagulation regime in future might therefore be best guided by global coagulation assessments such as thromboelastography.[19] A program of robust training for the nursing staff who would run the DIALIVE set up will be an important consideration to deliver safe treatment, as most of the sessions of DIALIVE in the present study were delivered by a dedicated team.

Table 3. Serious adverse events in the two groups.

Patient	Modified safety	Adverse event description	Treatment related	Study protocol procedure related	Severity	Causality	Between D1 and D10
DIALIVE SAEs: safety set (11 patients; 64.7%)							
1	Yes	Severe drop in platelets during first failed treatment session	Yes	Possibly	Severe	Possible	Yes
2	Yes	UTI by sensitive bacteria. AKI	No	No	Severe	Not related	No
	Yes	Bleed at dialysis line puncture site	Possibly	Possibly	Severe	Probable	Yes
	Yes	DIC with bleeding from dialysis line puncture site	Possibly	Possibly	Severe	Possible	Yes
	Yes	Sudden loss of consciousness followed by subdural hematoma. Concurrent seizures	No	No	Severe	Unlikely	Yes
3	No	Severe hypotension	No	No	Severe	Not related	Yes
	No	Multiorgan failure	No	No	Severe	Not related	Yes
4	Yes	Massive myocardial infarction	No	No	Severe	Not related	Yes
5	Yes	Hypocalcemia & hypophosphatemia	Yes	Yes	Severe	Related	Yes
6	Yes	Hypotension	Possibly	Possibly	Moderate	Probable	Yes
7	No	Hypotension	No	Yes	Moderate	Related	Yes
	No	DIC	Possibly	Possibly	Severe	Possible	Yes
	No	Septic shock	Yes	Yes	Severe	Probable	Yes
8	Yes	Anemia	Possibly	No	Severe	Possible	Yes
9	Yes	Bacteremia	No	Possibly	Severe	Not related	Yes
10	Yes	Hypotension	Possibly	Possibly	Moderate	Possible	Yes
	Yes	Multiorgan failure	No	No	Severe	Not related	No
11	Yes	UTI	No	No	Moderate	Not related	No
SOC SAEs: safety set (8 patients; 53.3%)							
1	Yes	Hepatic encephalopathy grade II	N/A	No	Severe	N/A	No
2	Yes	Asymptomatic bacterial infection, worsening in refractory septic shock	N/A	No	Severe	N/A	Yes
	Yes	Gastrointestinal bleeding worsening to hemorrhagic shock	N/A	No	Severe	N/A	Yes
3	Yes	Worsening liver disease	N/A	No	Severe	N/A	No
4	Yes	AKI	N/A	No	Moderate	N/A	Yes
	Yes	Cardiac arrest	N/A	No	Severe	N/A	Yes
	Yes	Multi organ failure as a result of progressive liver disease	N/A	No	Severe	N/A	No
	Yes	Upper gastro-intestinal bleed	N/A	No	Severe	N/A	Yes
5	Yes	*Klebsiella pneumonia* in blood culture	N/A	No	Severe	N/A	Yes
6	Yes	Patient transferred to local hospice for palliative care	N/A	No	Severe	N/A	No
7	Yes	Deterioration of portal flow	N/A	No	Moderate	N/A	No
8	Yes	Severe bleeding from puncture site of the central venous catheter	N/A	No	Severe	N/A	Yes
	Yes	Sepsis; fever >39 °C	N/A	No	Moderate	N/A	No

AKI, acute kidney injury; DIC, disseminated intravascular coagulation; N/A, not available; SAE, serious adverse event; SOC, standard of care; UTI, urinary tract infection.

The application of DIALIVE in clinical practice for the first time depicts evidence of a learning curve as evidenced by two deaths in the early phase of the study. These deaths occurred in hemodynamically unstable patients. Following extensive review by the DSMB, important necessary changes were made to patient management including the requirement to manage these patients in a high dependency area and for albumin replacement to occur during treatment and not at the end of the treatment session. In the modified-safety cohort, two other patients on DIALIVE died; one of whom had a CLIF-C ACLF score of 66, which is now widely regarded as a sub-group associated with extremely high risk of death and potential futility of ongoing ICU care.[1,2,6,7] Nevertheless, this patient tolerated the treatment well but died from sepsis due to a

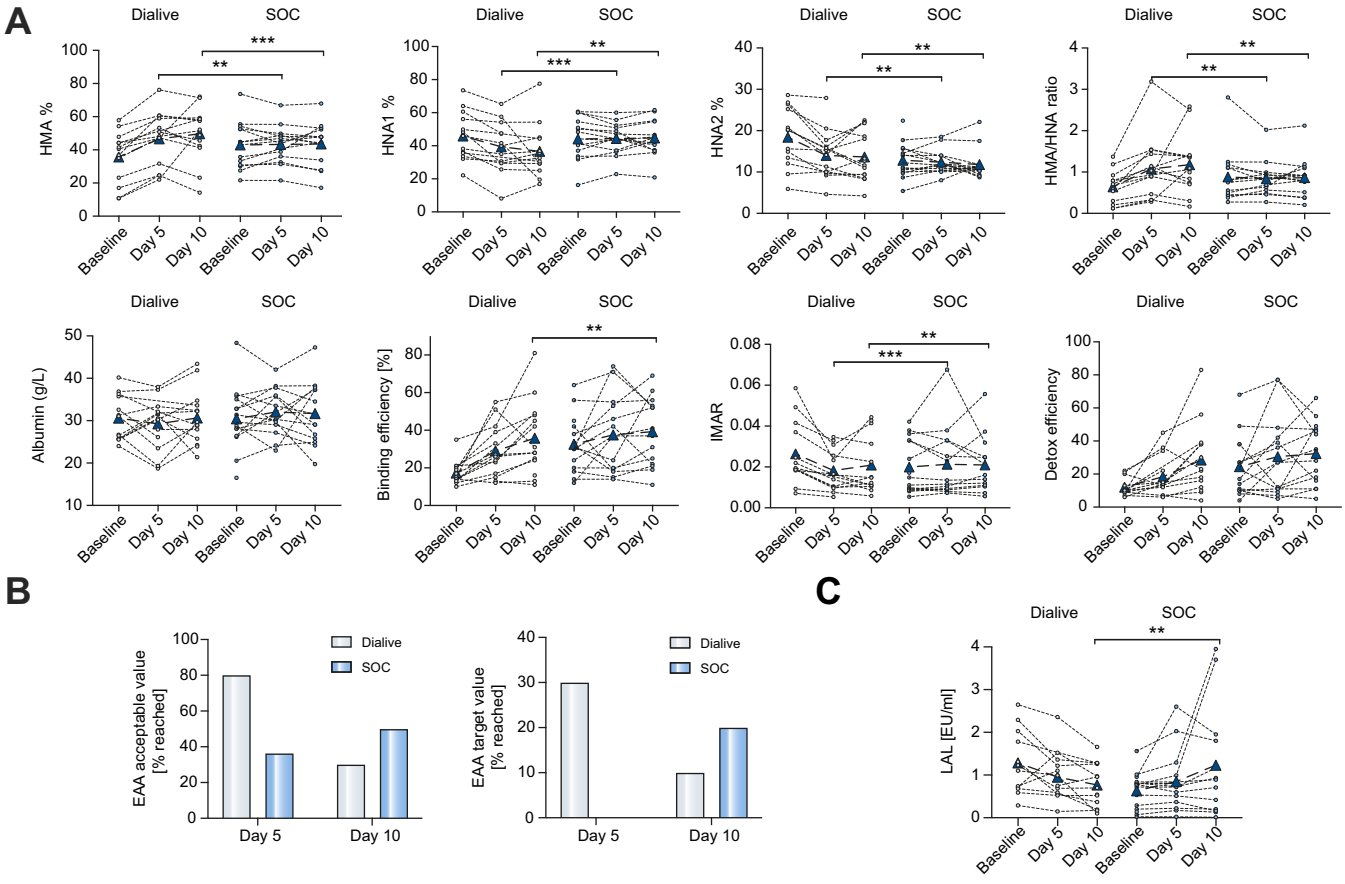

Fig. 1. Biomarkers of device performance in patients treated with DIALIVE or SOC. (A) These eight panels describe changes in albumin concentration and the functional domains that were measured in the DIALIVE and SOC groups at Days 0, 5 and 10. The data show that there was no significant difference in the albumin concentration either within the group nor between the groups. There was a significant increase in HMA and a reduction in HNA-1 and -2 both at Days 5 and 10 in the DIALIVE arm compared with the SOC arm, resulting in a significant increase in albumin redox status reflected by an increase in the HMA/HNA ratio at Days 5 and 10 respectively. There was a significant increase in the binding efficiency of albumin at Day 10 in the DIALIVE group compared with the SOC group, but detoxification efficiency did not change significantly. IMAR was significantly reduced in the DIALIVE group compared with the SOC group at both Days 5 and 10. (B,C) These three panels describe changes in markers of endotoxin activity. The results for endotoxin activity assays described here represent data from patients with a coefficient of variation <15%. Pre-specified target value was 40% and acceptable value was 20% (B). The second endotoxin measure used was the limulus amebocyte lysate assay. There was also a statistically significant advantage for DIALIVE at Day 10 (C). The analyses were performed using data from all patients from the modified safety population included in both the groups. MMRM analysis was performed to evaluate the statistically significant differences between groups (SOC and DIALIVE) at the main time-points including absolute values (Day 5 and Day 10). Reported *p* values, effect sizes and 95% CIs of time effect comparison obtained from MMRM for the absolute values adjusted by treatment, time (Baseline, Day 5 and Day 10) and interaction between time and treatment. *Describes statistically significant difference within the groups and between groups at specified time points [*p <0.05; **p <0.01; ***p <0.001]. Source data 1. HMA, human mercapt albumin; HNA, human non-mercapt albumin; IMAR, ischemia-modified albumin ratio; MMRM, mixed models for repeated measurements; SOC, standard of care.

previously undiagnosed osteomyelitis. The second death was precipitated by an acute myocardial infarction well after the end of DIALIVE treatment and was thought to be unrelated to DIALIVE treatment. All three deaths in the SOC group were thought to be liver related.

The treatment-emergent and the serious AE rate in the two groups was similar. Patients on DIALIVE had a greater incidence of thrombocytopenia, bleeding, and hypotension, which are not uncommon in critically ill patients receiving extracorporeal therapy.[8,9] Following protocol modification after the inclusion of Cohort 2, the incidence of hypotension was almost fully addressed. A further two milder episodes were corrected with prompt recognition and appropriate action around the time of starting DIALIVE therapy. The most frequent SAE in patients on SOC was infection, which is well-known to complicate the course of ACLF and is a major cause of death.[2] DIALIVE-treated patients had lower rates of new infections and this is likely secondary to attenuation/dampening of endotoxemia, which drives the risk of infection through its deleterious effects on neutrophil function.[15] In pre-clinical studies, DIALIVE has also been shown to restore neutrophil function.[12]

Albumin is the most abundant plasma protein in humans and has many pleotropic effects.[20,21] In ACLF, there is both a reduction in the quantity and function of the circulating albumin, which not only adversely impacts on its detoxification ability, but the oxidized forms act as pro-inflammatory species and contribute to systemic inflammation.[3,22] DIALIVE, by virtue of exchanging the dysfunctional albumin with bottled albumin, led to significantly improved albumin function. This was despite there being no difference in the concentration of circulating albumin between the DIALIVE and the SOC groups. During the 8-hour treatment period, about 40-50 g of albumin was

recovered from the effluent, which is roughly what was replaced. DIALIVE treatment resulted in an improvement in the thiol function, a reduction in the deleterious (HNA-1) and permanently damaged (HNA-2) fractions of albumin, binding and detoxification function and metal binding ability. It is important to note that this improved functionality was apparent even at Day 10, which is well beyond the scheduled treatment of 5 days, indicating perhaps that the sustained improvement might be a reflection of the modification of pathogenic factors responsible for albumin dysfunction.

Bacterial translocation is a particular feature of cirrhosis and ACLF, manifesting as an accumulation of PAMPs.[23,24] As many of these substances are ligands for the Toll-like receptor and inflammasome pathways, they can drive systemic inflammation. There is good evidence linking accumulation of lipopolysaccharides with systemic inflammation and risk of mortality in ACLF.[3,4,25] In *in vitro* studies, removal of endotoxin or its function, prevented neutrophil dysfunction.[15] DIALIVE was therefore specifically designed to remove endotoxin. In this study, a significantly greater reduction in the severity of endotoxemia, using the limulus amebocyte lysate assay, was observed in the DIALIVE-treated patients, an effect that was sustained at Day 10. It is difficult to draw conclusions from the results of the endotoxin activity assay as the data was analyzed in only about two-thirds of the patients in both groups due to the high coefficient of variation in the others. Nevertheless, DIALIVE treatment reached the pre-defined acceptable value for >20% reduction from baseline in 80% and 30% patients at 5 days and 10 days compared with 36% and 50% in the SOC group, respectively, but this was not statistically significant.

The most important observation of potential efficacy of DIALIVE was the significantly greater improvement in the liver, kidney, coagulation, and brain sub-scores of CLIF-OF scores compared with the SOC group at Day 10. Collectively, this resulted in a significant reduction in the CLIF-C OF score and the CLIF-C ACLF score, and a significantly reduced time to resolution of ACLF in the DIALIVE group. Additionally, a larger proportion of patients achieved ACLF resolution with DIALIVE treatment (43% *vs.* 27%). These data are important since ACLF resolution itself is a desirable clinical endpoint. Previous observations confirm that resolution of ACLF at Days 3-7 does translate into survival benefit at 28 and 90 days.[6] From the clinical standpoint, in addition to potentially improving survival, resolution of ACLF may enable patients to be discharged from the ICU or for bridging to transplantation in those that do not resolve ACLF completely.[26] In this small study, ICU stays between groups were similar.

MARS (molecular adsorbents recirculating systems) and Prometheus are extracorporeal liver assist devices using principles of albumin dialysis, which were tested in large clinical trials and shown not to reduce mortality. Extracorporeal cellular therapy used hepatoblastoma-derived cells in the dialysis circuit to treat patients with severe alcohol-related hepatitis but again failed to show a survival benefit.[10] DIALIVE is very different to these devices as it has been designed to directly impact some of the known pathophysiological mechanisms of ACLF and it does so by exchanging dysfunctional albumin and removing inflammatory mediators (PAMPs and DAMPS).

The ACLF-related biomarker data provide insights into the mechanisms by which DIALIVE could exert positive clinical effects. First, the effect of DIALIVE on the severity of systemic inflammation is clear from trends towards reduction in many of the cytokines measured, of which the most significant was IL-8. A change in IL-8 has been shown to be associated with resolution of ACLF.[3] Second, the data showed a significant and sustained effect of DIALIVE on markers of cell death, which are known to be elevated in ACLF.[16] Both the M30 component of cytokeratin-18, a marker of apoptosis and RIPK3, a marker of necroptosis were significantly reduced in the DIALIVE-treated patients. Third, together with the observed reduction in PAMPs described above, these translated into a significantly lower burden of ligands that stimulate the TLR4 or the inflammasome pathways. Fourth, there was a significant effect of DIALIVE on markers of endothelial function, which is known to be dysfunctional in ACLF.[27]

A correlation analysis of the factors associated with ACLF resolution with DIALIVE suggested a multimodal effect on all categories of the pathophysiological variables known to be associated with the pathogenesis of ACLF. When patients from both groups were combined, ACLF resolution was again shown to be associated with modulation of each of the pathways studied rather than a predominance of any single pathway. This observation is largely in keeping with previous data, which have shown similar associations with ACLF resolution and suggests that any biomarker(s) for the early prediction of resolution of ACLF will require a panel combining markers representing multiple pathways.[3–5,16] The data showing that an increase in CCL5/Rantes, which is a chemokine, was associated with resolution of ACLF in the SOC group and when both groups were combined, seems paradoxical. Previous studies in patients with alcohol-related liver disease have shown elevated levels but whether this increase is pathological or compensatory is unknown.[28] The multidimensional effect of DIALIVE does not allow for identification of a particular pathway but confirms the importance of albumin dysfunction, and PAMPs and DAMPs, in the pathogenesis of ACLF, as these were the main variables directly targeted by DIALIVE.

This study faces the challenges and therefore the limitations of a first-in-man study of a new therapeutic approach to treat ACLF. First, accrual of patients for the trials was challenging given restrictive eligibility criteria. Second, given the multicenter nature of the study, SOC was difficult to establish particularly during the inclusion of the first 12 patients. Third, although there were positive, statistically significant efficacy data, the results should be interpreted cautiously considering that the study was designed to evaluate safety, the analyses are *post hoc* and the sample size is relatively small. Fourth, even though ACLF is heterogenous, the inclusion of only patients with a clinical diagnosis of alcohol-related cirrhosis allowed for a degree of homogenization. Therefore, generalization to other etiologies and precipitants must be evaluated on a case-by-case basis. Fifth, it is difficult to rule out an element of bias as this was an unblinded study, but there are serious ethical issues around using sham therapy. Sixth, the secondary endpoints of duration of ICU and hospitalization were not possible to measure accurately due to vast differences in practice. Finally, with the limited number of patients per group, it is difficult to identify

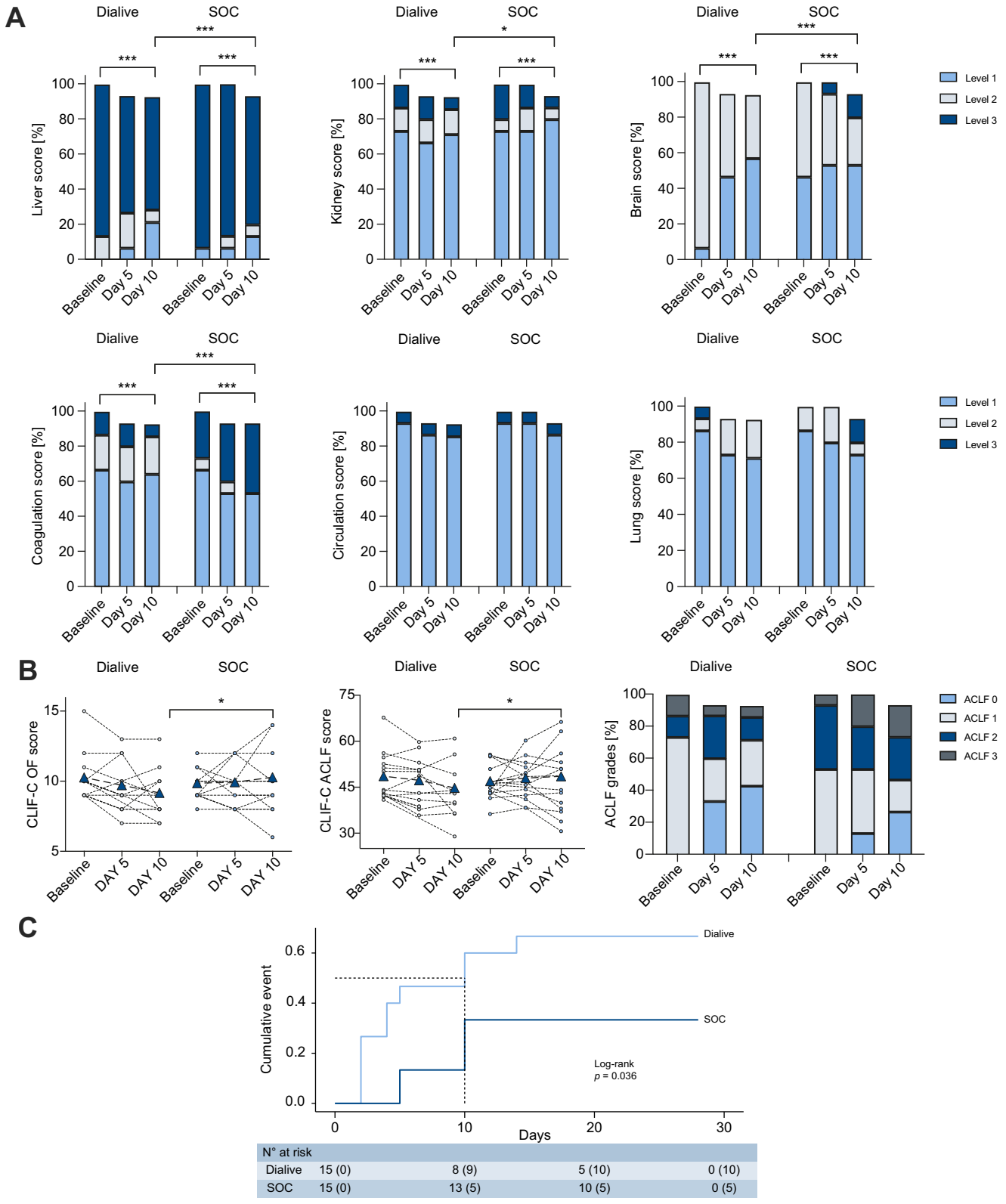

Fig. 2. Clinical effect of DIALIVE or SOC on severity of ACLF and its resolution. (A) These six panels describe the effect of DIALIVE or SOC on individual organ functions There were significant improvements in the liver, kidney, coagulation, and brain sub scores in both groups but the changes in each of these subscores was significantly greater in the DIALIVE group at Day 10. (B) Significantly greater improvements in the CLIF-OF and CLIF-C ACLF scores were observed in the DIALIVE group at Day 10. Although ACLF grades were not statistically different between groups, there were greater proportion of patients in whom ACLF resolved at Day 10 (42.9% *vs.* 26.7%; $p = 0.450$) in the DIALIVE group, which was not statistically significant. (C) The time to resolution of ACLF was also significantly faster (log-rank $p = 0.036$). The analyses were performed using data from all patients from the modified safety population included in both the groups. MMRM analysis was performed to evaluate the statistically significant differences between groups (SOC and DIALIVE) at the main time-points (Day 5 and Day 10). Reported *p* values, effect sizes and

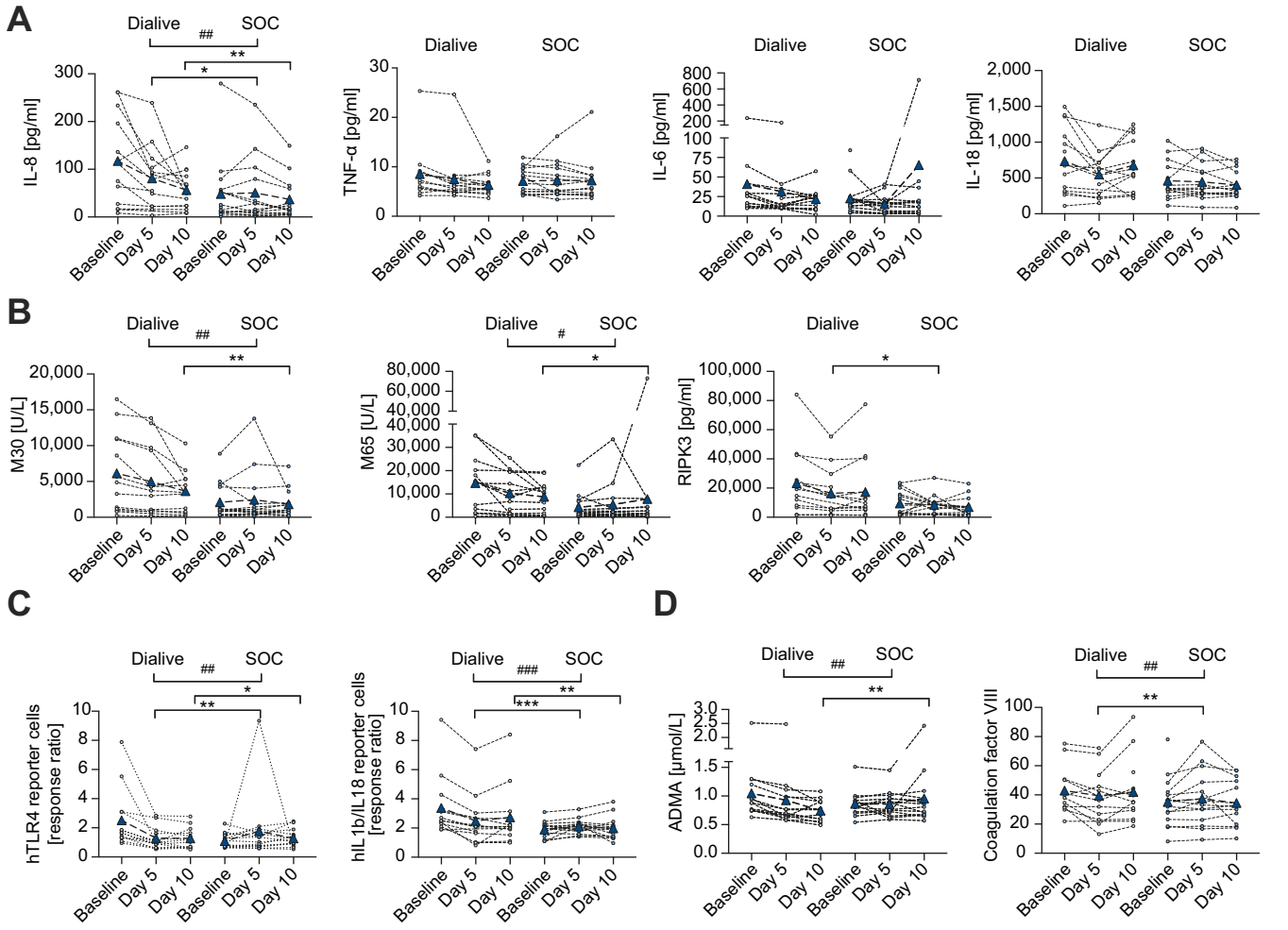

Fig. 3. Selected biomarkers assessing the impact of DIALIVE or SOC on the pathophysiological factors associated with ACLF. (A) These four panels show changes in markers of the key cytokines and chemokines associated with ACLF. DIALIVE significantly lowered IL-8 at both Day 5 and Day 10 compared with SOC, resulting in a significant overall treatment effect. For TNF-α, IL-6 and IL-18 there were trends to lower levels, but no statistical significance was observed. (B) These three panels describe markers of cell death. For the M30 component of cytokeratin-18, a marker of apoptosis, there was a significant treatment effect overall and a significant reduction at Day 10 compared to the SOC group. For the M65 component of cytokeratin-18, there was a significant treatment effect overall and a significant advantage for DIALIVE at Day 10. For RIPK3 there was a significant advantage for DIALIVE at Day 5. (C) The first two panels show the effect of incubation of the patient's plasma with reporter cell lines that would become activated by TLR4 or inflammasome ligands. There was a significant treatment effect overall with significant reduction for the DIALIVE group at both Day 5 and Day 10 when the patient's plasma was incubated with a TLR4 reporter cell line. Similarly, there was an overall significant treatment effect when the patient's plasma was incubated with the IL-1β/IL-18 inflammasome cell line. There were significant advantages for DIALIVE at both Day 5 and Day 10. (D) The third and fourth panels show biomarkers of endothelial dysfunction. For ADMA there was a significant treatment effect overall with a significant reduction in the DIALIVE group at Day 10. For Factor VIII, there was a significant treatment effect overall in favor of DIALIVE with a significant reduction observed at Day 5. The analyses were performed using data from all patients from the modified safety population included in both the groups. MMRM analysis was performed to evaluate the statistically significant differences between groups (SOC and DIALIVE) at the main time-points (Day 5 and Day 10). Reported *p* values, effect sizes and 95% CIs of time effect comparisons obtained from MMRM for the absolute values adjusted by treatment, time and interaction between time and treatment. Overall treatment effect was calculated using absolute differences adjusted by treatment and interaction between both. *Describes statistically significant difference with the groups and between groups at specified time points [*p <0.05; **p <0.01; ***p <0.001]. #Describes overall treatment effect [#p <0.05; ##p <p.01]. Source data 3. ACLF, acute-on-chronic liver failure; ADMA, asymmetric dimethylarginine; MMRM, mixed models for repeated measurements; SOC, standard of care.

any safety signal. Thus, any favorable claims made in the study will need to be verified in a future study involving powered safety and efficacy endpoints.

In conclusion, the data from this study suggests that DIALIVE is likely to be safe with careful patient management and cautious monitoring in hemodynamically unstable patients. DIALIVE achieves its aims of reducing endotoxin and improving albumin

95% CIs of time effect and within treatment comparison obtained from MMRM for the absolute values adjusted by treatment, time and interaction between time and treatment. Overall treatment effect was calculated from absolute differences adjusted by treatment and interaction between both. Individual organ scores and ACLF grade were evaluated using cumulative link mixed models with Laplace approximation adjusted by treatment, time and interaction between time and treatment. Kaplan-Meier curves were constructed to determine differences between groups in the resolution of ACLF and a log-rank test was used for survival comparison. *Describes statistically significant differences within the groups and between groups at specified time points [*p <0.05; **p <0.01; ***p <0.001]. #Describes overall treatment effect [#p <0.05; ##p <0.01]. Source data 2. ACLF, acute-on-chronic liver failure; CLIF-C, Chronic Liver Failure Consortium; MMRM, mixed models for repeated measurements; OF, organ failure; SOC, standard of care.

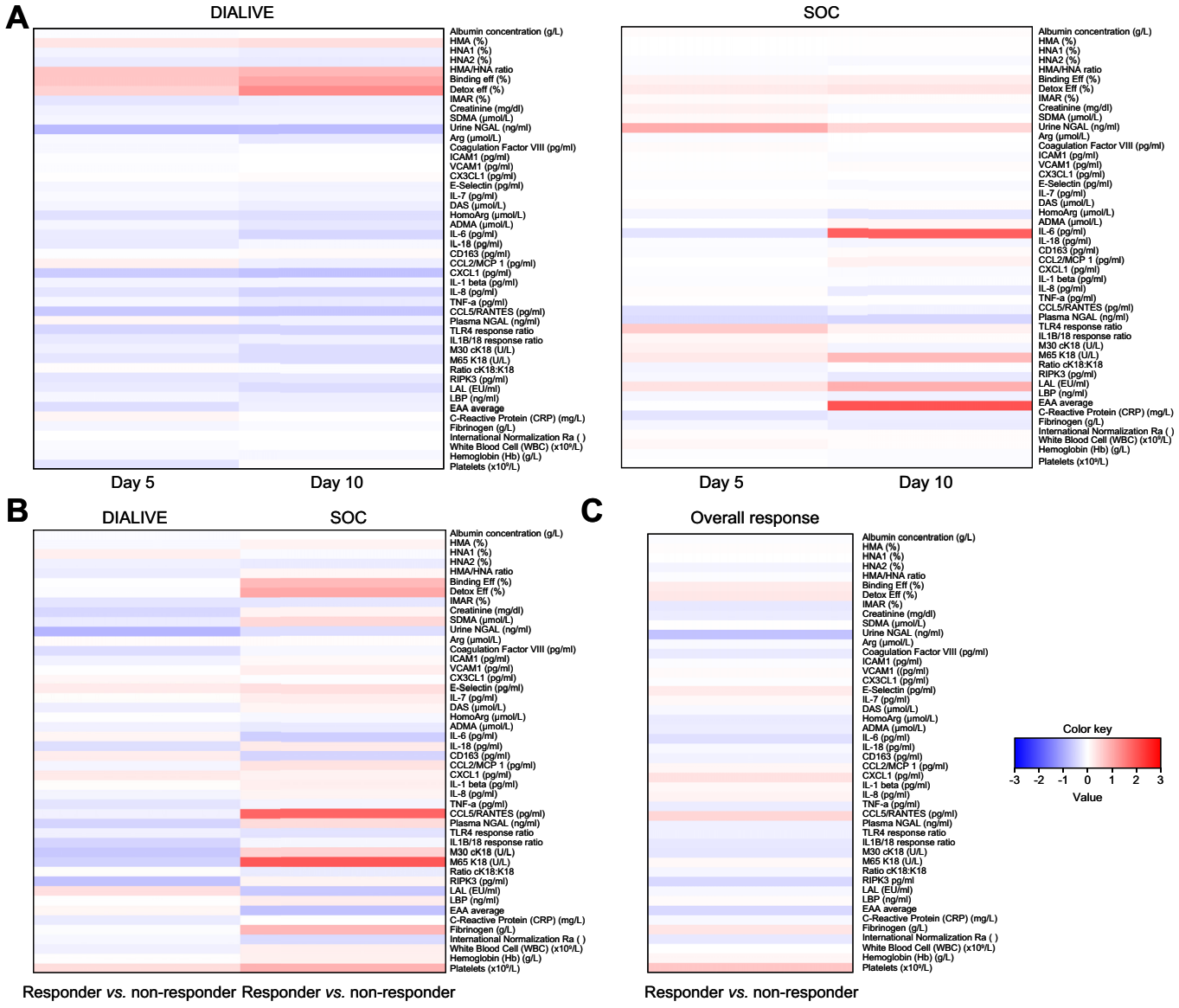

Fig. 4. Relationship of the device performance and pathophysiology-related biomarkers to resolution of ACLF. (A) This describes trends of changes from baseline in all the biomarkers measured in the patients treated with DIALIVE and SOC at Days 5 and 10. Statistically significant changes in HMA, HNA-2 and IL-8 at both Day 5 and 10 were observed. Furthermore, statistically significant changes at only Day 5 were observed in IL-18, IL-1β, IL-1β/IL-18 inflammasome cell line response, RIPK3 and platelets. Statistically significant changes at Day 10 alone were observed in HNA-1, HMA/HNA ratio and M30 component of cytokeratin-18. More variable results of the biomarkers were observed in the patients treated with SOC. Red indicates higher values at Day 5 or Day 10 compared to baseline, blue otherwise. (B) This describes trends of changes from baseline and Day 10 in all the biomarkers measured and their relationship with resolution of ACLF (responder *vs.* non-responder) in the patients treated with DIALIVE or SOC at Day 10. Resolution of ACLF in the DIALIVE group was associated with trends to improvements in most of the biomarkers measured with significant changes in coagulation factor VIII, IL-18, M30 component of cytokeratin-18 and RIPK3. In the SOC group, there was an apparently paradoxical relationship with CCL5/Rantes and M65 component of cytokeratin-18 being associated with resolution of ACLF. A reduction in international normalized ratio was associated with resolution of ACLF. The color scale for each parameter has been calculated by subtracting the mean value in non-responders from the mean value in responders and then dividing by the mean in non-responders. Red indicates higher values in the responder group, blue otherwise. (C) This describes trends of changes from baseline and Day 10 in all the biomarkers measured and their relationship with resolution of ACLF (responder *vs.* non-responder) in the patients treated with either DIALIVE or SOC at Day 10. The data show that a reduction in coagulation factor VIII, IL-7, IL-18, RIPK3 and international normalized ratio and an increase in CCL5/Rantes were associated with resolution of ACLF. The color scale for each parameter has been calculated by subtracting the mean value in non-responders from the mean value in responders and then dividing by the mean in non-responders. Red indicates higher values in the responder group, blue otherwise. Source data 1 and 3. Also, Table S6. ACLF, acute-on-chronic liver failure; HMA, human mercapt albumin; HNA, human non-mercapt albumin; MMRM, mixed models for repeated measurements; SOC, standard of care. (This figure appears in color on the web.)

function, which impacts positively on organ function, allowing for a greater proportion of patients to resolve ACLF with greater rapidity. Given these early data, it is important to validate these findings in larger series of adequately powered clinical trials.

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Abbreviations

ACLF, acute-on-chronic liver failure; ADMA, asymmetric dimethylarginine; AEs, adverse events; AKI, acute kidney injury; CLIF-C, Chronic Liver Failure Consortium; DSMB, Data Safety Monitoring Board; EASL-CLIF, European Association for the Study of Liver diseases – Chronic Liver Failure; HMA, human mercaptalbumin; HNA, human non-mercapt albumin; ICU, intensive care unit; MMRM, mixed models for repeated measurements; OF, organ failure; SAEs, serious adverse events; SOC, standard of care; RIPK3, receptor-interacting serine/threonine-protein kinase 3; TLR4, Toll-like 4 receptor.

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Conflicts of interest

Rajiv Jalan is the inventor of OPA, which has been patented by UCL and licensed to Mallinckrodt Pharma. He is also the founder of Yaqrit Discovery (the company that holds the intellectual property for DIALIVE), a spin out company from University College London, Hepyx Limited and Cyberliver. He had research collaborations with Yaqrit Discovery. Nathan Davies, Phil Lavin, Rajeshwar P Mookerjee, Daniel Green are share holders in Yaqrit Discovery. Carrie Morgan is employed by Yaqrit Discovery. Katja Waterstradt is employed by MedInnovation. Jan Stange is the Founder of Albutech GmBh. Jaak Minten is employed by Fakkel. Moises Sanchez is employed by IBM. The rest of the authors do not declare any conflict of interest.

Please refer to the accompanying ICMJE disclosure forms for further details.

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Data availabilty statement

The data shown in this article are available from the corresponding authors upon a reasonable request.

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Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhep.2023.03.013.

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Author names in bold designate shared co-first authorship.

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