A phase II study of human allogeneic liver-derived progenitor cell therapy for acute-on-chronic liver failure and acute decompensation

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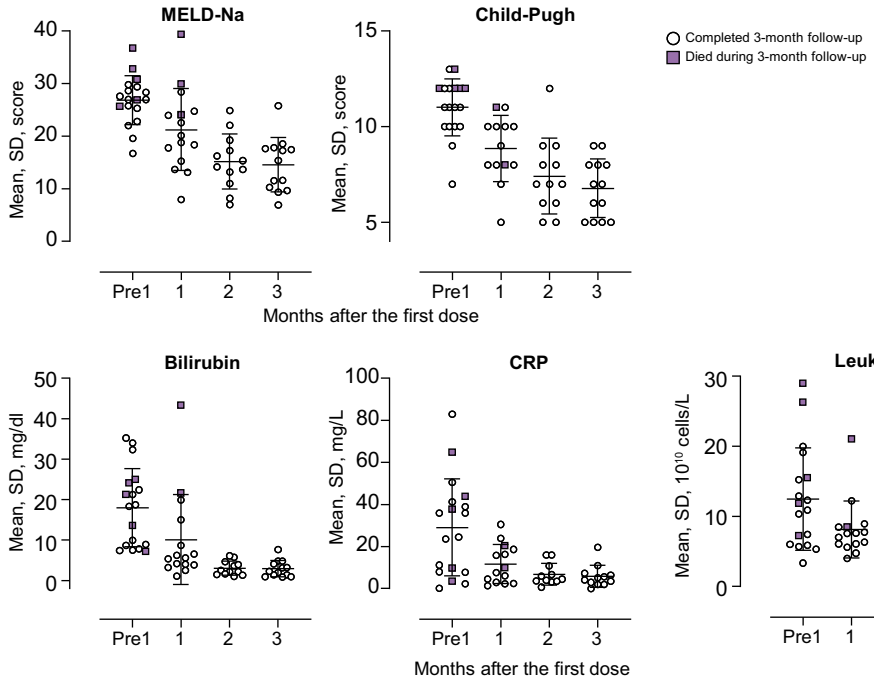
Graphical abstract



Highlights

- In cirrhosis, ACLF and AD are acute phases associated with high risk of death.
- HALPC therapy is classified as an ATMP that can potentially treat ACLF/AD.
- Up to 2 i.v. infusions of 0.6–1.2×10^6 HALPC/kg body weight appeared safe.
- Post HALPC infusion, markers of systemic inflammation and altered liver function decreased gradually for the surviving patients.
- Day-28 and Month-3 survival rates were 83% and 71%; and no patient had ACLF at Month 3.

Lay summary

Patients with liver cirrhosis may suffer from the rapid onset of organ failure or multiple organ failure associated with a high risk of death in the short term. This clinical study of 24 patients suggests that an advanced therapy based on the intravenous infusion of low doses of human allogeneic liver-derived progenitor cells is safe and supports the next phase of clinical development of this type of therapy.

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Research article

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A phase II study of human allogeneic liver-derived progenitor cell therapy for acute-on-chronic liver failure and acute decompensation

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Background & Aims: Human allogeneic liver-derived progenitor cells (HALPC, HepaStem®; Promethera Biosciences, Mont-Saint-Guibert, Belgium) are an advanced therapy medicinal product that could potentially alleviate systemic inflammation and ameliorate liver function in patients with acute-on-chronic liver failure (ACLF) or acute decompensation of cirrhosis (AD).
Methods: This open-label phase II study was conducted in 9 centres in Belgium, Spain, and Bulgaria between 2016 and 2019. The primary objective was to assess the safety of HALPC therapy up to Day 28 and the secondary objectives were to assess its safety and preliminary efficacy up to Month 3.
Results: The 24 treated patients (mean age: 51 years) were mostly male with an alcoholic cirrhosis. On pre-infusion Day 1, 15 patients had ACLF and 9 patients had AD. Two of the 3 initial patients treated with high HALPC doses ($\sim 5\times10^6$ cells/kg body weight [BW]) had severe adverse bleeding events attributed to treatment. In 21 patients subsequently treated with lower HALPC doses (0.6 or 1.2×10^6 cells/kg BW, 1 or 2 times 7 days apart), no serious adverse events were related to treatment, and the other adverse events were in line with those expected in patients with ACLF and AD. Overall, markers of systemic inflammation and altered liver function decreased gradually for the surviving patients. The Day-28 and Month-3 survival rates were 83% (20/24) and 71% (17/24), and at Month 3, no patient had ACLF.
Conclusions: The treatment of patients with ACLF or AD with up to 2 doses of 1.2×10^6 HALPC/kg BW appeared safe. The results of this study support the initiation of a proof-of-concept study in a larger cohort of patients with ACLF to further confirm the safety and evaluate the efficacy of HALPC therapy.
Clinical Trials Registration: EudraCT 2016-001177-32.
Lay summary: Patients with liver cirrhosis may suffer from the rapid onset of organ failure or multiple organ failure associated with a high risk of death in the short term. This clinical study of 24 patients suggests that an advanced therapy based on the intravenous infusion of low doses of human allogeneic liver-derived progenitor cells is safe and supports the next phase of clinical development of this type of therapy.

Introduction

The acute development of 1 or more major complications of cirrhosis can be classified as acute decompensation of liver cirrhosis (AD) or acute-on-chronic liver failure (ACLF). Although the definitions of AD and ACLF can differ geographically, overall, these syndromes are characterised by single or multiple organ failure and a high risk of mortality.[1–3] Notably, ACLF is also a condition linked with systemic inflammation and immune dysfunction.[4–7] The Day-28 and Month-3 transplant-free mortality rates have been estimated at, respectively, 32.8% and 51.2% in ACLF patients, and 1.9% and 9.8% in patients with AD but without ACLF.[1] When stratified by total bilirubin, the Day-28 and Month-3 overall mortality rates have been estimated at,

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respectively, 26.4% and 39.4% in patients with ACLF with total bilirubin <5 mg/dl, and 63.7% and 76.3% in those with total bilirubin ≥5 mg/dl.[8]

ACLF can occur in 3–6% of patients with cirrhosis,[2] and the prevalence of ACLF in hospitalised patients with AD ranges from 24% to 34%.[2,9,10] ACLF can be classified into 3 grades, primarily based on the number of organ failures and with higher grading associated with higher mortality.[1–3]

At present, besides supportive care,[3,11] there is no specific treatment for ACLF that improves survival. Supportive care is symptom-dependent and includes antibiotics for infections, corticosteroids for severe alcoholic hepatitis, lactulose and rifaximin for encephalopathy, vasopressors and albumin for hepatorenal syndrome. The Molecular Adsorbent Recirculating System (MARS) and other artificial liver support devices can improve cerebral and renal function but not ACLF prognosis.[12,13] Circulatory support with vasopressors and intravenous (i.v.) albumin infusion, which improves renal function, also does not appear to improve survival.[14] Liver transplantation represents the only definitive therapeutic option for patients with ACLF. However, in contrast to patients with acute liver failure, most patients with ACLF cannot be listed on the high-urgency transplantation list, owing to advanced age, active alcoholism, uncontrolled infections, and multiple organ failure. Only 10–25% of patients with ACLF receive transplants and 50–70% of the patients down for transplant surgery die on the waiting list.[15] Moreover, ACLF represents a large healthcare and economic burden as a result of prolonged hospitalisation and resource-intensive treatment. Although defined differently from the European Association for the Study of Chronic Liver Failure (EASL-CLIF) criteria, the number of ACLF hospitalisations in the USA rapidly increased by 6-fold during 10 years (2001–2011), leading to a 5-fold higher cost.[16]

The limited access to transplantation, as well as the absence of approved products, result in a persisting high unmet medical need for ACLF. Accumulating preclinical and clinical evidence supports the therapeutic potential of bone-marrow-derived mesenchymal stem cells (MSC) in various immune-mediated diseases.[17] These multipotent cells can differentiate into a variety of cells of the mesodermal lineage, but their most relevant medicinal properties are related to their potent immunomodulatory properties.[18] Umbilical or bone-marrow-derived MSC have been used to treat patients with ACLF, and were associated in one study with a decrease in the model for end-stage liver disease (MELD) score, total bilirubin, and alanine aminotransferase;[19] and in another study with a significant increase in the 24-week survival rate, an improvement of liver function and a lower incidence of severe infections.[20]

Human allogeneic liver-derived progenitor cells (HALPC) therapy is classified as an advanced therapy medicinal product (ATMP) by the European Medicines Agency and represents an alternative potential stem cell treatment for ACLF. HALPC are derived from the parenchymal fraction of healthy human liver tissue.[21] HALPC have a liver-specific homing capacity after peripheral i.v. infusion and have immunomodulatory and anti-fibrotic properties.[22–26] The safety profile of HALPC therapy has been extensively investigated in preclinical studies and HALPC therapy has been safely administered in children with inborn errors of metabolism.[27,28]

Here, we report the results of a phase II clinical study of HALPC therapy in cirrhotic patients with ACLF or with AD at risk of developing ACLF. The primary objective of the study was to assess the safety of different HALPC regimens up to Day 28; and the secondary objectives were to evaluate the clinical and biological preliminary efficacy of the therapy, and to further assess its safety up to Month 3.

Patients and methods

Study design and participants

This open-label study was carried out in 9 centres in Belgium, Spain, and Bulgaria between December 2016 and October 2019. The protocol, its amendments, and other relevant study documentation were approved by an independent ethics committee in each participating country, and the study was conducted in accordance with the Guideline for Good Clinical Practice of the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use,[29] the Declaration of Helsinki (2013)[30] and all applicable regulatory requirements. HALPC (HepaStem®) were obtained from the sponsor, Promethera Biosciences (Mont-Saint-Guibert, Belgium), and produced with an agreement of the Belgian Ministry of Health. Human tissue sourcing was collected and provided as sourcing material by the hepatic tissue bank of the Cliniques Universitaires Saint-Luc, Brussels, Belgium, and by Promethera LLC, Durham NC, USA, collecting organs from US organ procurement organisations. All donors had not opted out from organ and tissue donation. Liver tissue was only obtained from organs or organ segments that could not be used for liver transplantation. All patients screened for the study provided written informed consent.

Procedures

Initially, eligible patients had Grade 1 or Grade 2 ACLF (based on the EASL-CLIF criteria) and the planned dose regimen was four infusions of 250×10^6 cells/dose. However, the protocol was amended after a temporary halt of the study before which 3 patients had been treated. In the amended protocol, eligible patients had (i) ACLF, or (ii) AD with the risk of developing ACLF and an international normalised ratio (INR) ≥1.2 and <2 (this range was later changed to INR ≥2). The dose and frequency of infusions were changed and adapted to the patient's body weight (BW) to 0.6 or 1.2×10^6 cells/kg BW, administered either as 1 (initially) or 2 infusions, 7 days apart.

The main exclusion criteria included: (i) thrombosis of the portal vein; (ii) ongoing uncontrolled bleeding; (iii) septic shock or non-controlled bacterial infection; (iv) circulatory failure; (v) mechanical ventilation as a result of respiratory failure; (vi) treatment with corticosteroids for acute liver disease less than 1 day before screening; (vii) previous organ transplantation and/or ongoing immunosuppressive treatments; (viii) postoperative decompensation after hepatectomy; (ix) major surgeries/invasive procedures within 4 weeks before HALPC infusion; and (x) MELD-Na score >35.

The screening period lasted up to 7 days (Fig. 1A). Patients were hospitalised in intensive care, intermediate, or standard units, depending on the severity of the patient's disease. During the screening period, the patient's comprehensive medical history was recorded, such as background condition leading to cirrhosis, possible previous episode(s) of AD/ACLF, and factor(s) triggering AD/ACLF. The active study period lasted 28 days (±2 days) and was divided into 2 periods; the treatment period during which HALPC therapy was administered, and the surveillance period. Patients were hospitalised during the screening

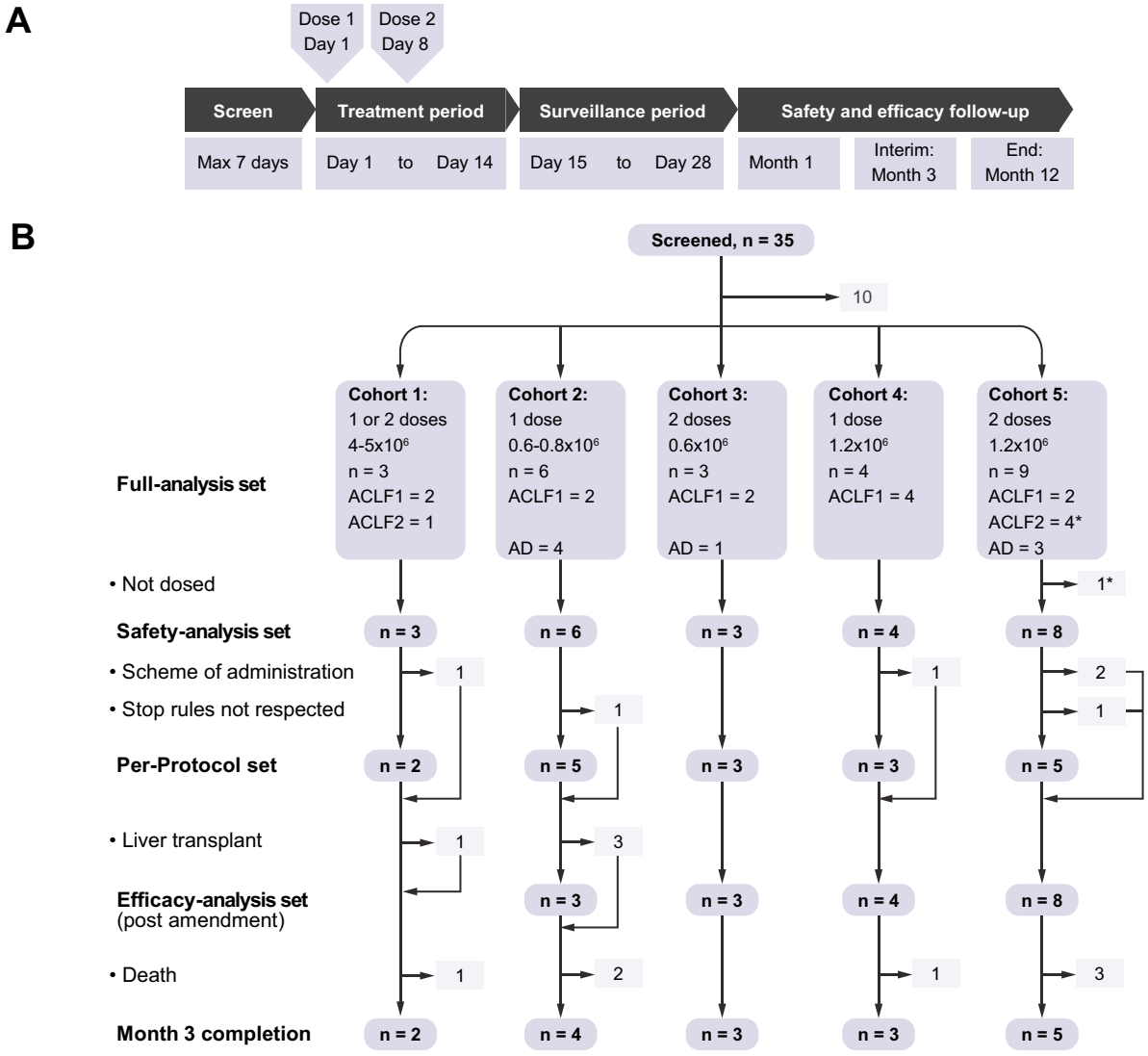

Fig. 1. Study design and patient disposition. (A) Schematic representation of the study design in accordance with the amended protocol. This article reports the study results of the interim analysis at Month 3. (B) Schematic description of patient disposition and allocation to cohorts and analysis sets. *Note that the AD/ACLF grades are for diagnoses based on parameters assessed at screening; for the patient in Cohort 5 who was not dosed, the diagnosis was Grade 2 ACLF at screening. ACLF, acute-on-chronic liver failure; AD, acute decompensation of liver cirrhosis.

and the treatment periods. Study visits were performed on Days 1, 4, 8, 12, and 14 during the treatment period, and on Days 21 and 28 during the surveillance period. Subsequently, patients entered the long-term follow-up period, including study visits at Months 2 and 3 for the analysis of the study. All patients received the same standard of care for liver disease throughout the study. In addition to receiving 1 bolus of glucocorticoids before HALPC infusion (see below), 8 patients received glucocorticoids before screening for severe alcoholic hepatitis, and 9 other patients received glucocorticoids after the screening assessments or later for liver transplantation or complications of ACLF.

Outcomes

The primary outcome was the occurrence of adverse events (AEs) up to Day 28, graded by seriousness, severity, relationship to HALPC therapy or its administration, and included clinically significant changes in clinical examinations, vital signs, laboratory tests, cardiac Doppler ultrasound, and abdominal echography with Doppler. The relationship to HALPC therapy or study procedures was based on the investigator's assessment and confirmed by the safety monitoring committee and the sponsor's pharmacovigilance unit, and in line with the guideline for ATMPs.[31]

The secondary outcomes for preliminary efficacy assessments were at Day 28 and Month 3: (i) features of systemic inflammation such as C-reactive protein (CRP) level and leucocyte count, (ii) other serum biochemistry and haematological parameters, and (iii) disease scores (CLIF-OF, CLIF-C ACLF, CLIF ACLF grade, CLIF-C AD, MELD-Na, and Child-Pugh); and up to Month 3: (i) the occurrence of a fatal event, and (ii) the occurrence of a liver transplantation.

Table 1. Demographic characteristics at baseline (Day 1).

Parameter	N	Value
Age* ± SD, years	24	50.5 ± 9.2
Female/male, n	24	7/17
Aetiology*	24	
Alcoholic cirrhosis, n		23
Ulcero-haemorrhagic rectocolitis complication, n		1
ACLF Grade 1/Grade 2, n	24	10/5
AD, n	24	9
Triggering factors* of AD/ACLF		
Active alcoholism/infection/hepatitis/other, n	24	20/3/1/3
Biochemistry mean ± SD (minimum–maximum)		
Creatinine (mg/dl)	24	1.0 ± 0.47 (0.33–2.4)
INR	23	2.0 ± 0.5 (1.2–3.0)
MELD-Na†	24	27 ± 4.2 (17–37)
Bilirubin (mg/dl)	24	20 ± 10 (7.2–35)
Platelets (10^9/L)	23	132 ± 82 (40–292)
Fibrinogen (g/L)	21	2.4 ± 1.1 (0.88–5.0)
Leucocytes (10^9/L)	24	12 ± 6.9 (2.0–29)
Neutrophils (10^9/L)	17	10 ± 7.0 (0.88–26)
CRP (mg/L)	23	29 ± 21 (0.23–83)

AD, acute decompensation of liver cirrhosis; CRP, C-reactive protein; INR, international normalised ratio; MELD-Na, MELD, model for end-stage liver disease-sodium; SD: standard deviation.
* Parameters reported at screening; other parameters reported at baseline (Day 1).
† Note that no enrolled patient had a MELD-Na score >35 at screening.

The secondary outcome for safety was the occurrence of AEs of special interest (AESIs), including serious AEs (SAEs) with fatal outcome, malignancies, liver transplantations, hospitalisations for new episodes of ACLF, and AEs assessed by the investigator as possibly related to HALPC therapy.

HALPC reconstitution and infusion

HALPC product (HepaStem®) was shipped in dry shippers filled with liquid nitrogen. Within 2 h before administration, HALPC were reconstituted in a diluent supplied by Promethera Biosciences to 5×10^6 cells/ml. Between 15 and 30 min before each infusion, a single bolus of 100 mg of hydrocortisone or equivalent was administered to the patient. HALPC were administered to the patient by i.v. infusion with gentle agitation of the syringe containing the cells during the infusion.

On the same day before each infusion, a physical examination, an evaluation of vital signs, and blood tests were performed. Coagulation parameters, including INR, fibrinogen, platelets, activated partial thromboplastin time, thromboelastography (TEG), and thrombin generation test (TGT) were performed before and at certain intervals up to 72 h after infusion. During the infusion, the patient was continuously monitored for potential AEs. On the other days during the hospital stay, patients were followed according to usual practice.

Statistical analysis and sample size

The sample size was typical of a safety and preliminary efficacy study evaluating cell-based ATMPs. The analyses were descriptive and no formal hypotheses were assessed. Descriptive statistics for quantitative variables consisted of number of observed values, number of missing observations, mean, median, standard deviation (SD), minimum, and maximum. Categorical parameters were summarised using absolute (number of participants) and relative (percentages calculated based on non-missing data) frequencies.

Results

Demography, disposition, and protocol amendment

A total of 25 patients were enrolled and 24 patients received the study treatment (Fig. 1B). Of the 24 treated patients (mean age: 51 years, mostly male; Table 1), 23 patients had alcoholic cirrhosis and 1 patient had biliary cirrhosis as a result of sclerosing cholangitis associated with ulcerative colitis. At screening, 12 patients had Grade 1 ACLF and 4 patients had Grade 2 ACLF (Fig. 1B). At baseline (Day 1), 10 patients had Grade 1 ACLF, 5 patients had Grade 2 ACLF, and 9 patients had AD (Table 1). All patients had bilirubin levels ≥7.2 mg/dl (mean 20 mg/dl; maximum 35 mg/dl; Table 1). The triggering factors for AD or ACLF were reported as alcohol intake in 20 cases, infection in 3 cases, alcoholic hepatitis in 1 case, and listed as 'other' in 3 cases.

A sentinel of 3 patients were treated first. The actual quantity of cells injected in the first patient (with Grade 2 ACLF) was negligible owing to the product sedimenting in the syringe. For subsequent patients, gentle agitation of the syringe during infusion secured proper infusion.

Each of the other 2 patients in the sentinel (Grade 2 ACLF and AD at infusion) received approximately 5×10^6 cells/kg BW and both reported suspected unexpected serious adverse reactions (SUSARs). One SUSAR was severe bleeding at a jugular puncture site after the second infusion, and the other SUSAR consisted of

Table 2. Adverse events (AEs) occurring during the first 28 days of the study (Safety Analysis set).

	AEs occurring during the active study period (within 28 days after HALPC infusion)											
	Cohort 1 (N = 3)		Cohort 2 (N = 6)		Cohort 3 (N = 3)		Cohort 4 (N = 4)		Cohort 5 (N = 8)		Total (N = 24)	
	n (%)	NoE	n (%)	NoE	n (%)	NoE	n (%)	NoE	n (%)	NoE	n (%)	NoE
Any AE	3 (100)	11	6 (100)	46	1 (33)	1	4 (100)	23	7 (88)	40	21 (88)	121
Related to HALPC therapy	2 (67)	4	2 (33)	2	0 (0)	0	0 (0)	0	0 (0)	0	4 (17)	6
Related to study procedure	2 (67)	4	1 (17)	1	0 (0)	0	0 (0)	0	0 (0)	0	3 (12)	5
Leading to study stop	2 (67)	4	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)	0	2 (8)	4
Any severe AE	3 (100)	5	3 (50)	9	0 (0)	0	2 (50)	2	3 (38)	9	11 (46)	25
Any serious AE	3 (100)	5	3 (50)	7	0 (0)	0	1 (25)	1	3 (38)	5	10 (42)	18
Related to HALPC therapy	2 (67)	4	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)	0	2 (8)	4
Related to study procedure	2 (67)	4	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)	0	2 (8)	4
Leading to study stop	2 (67)	4	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)	0	2 (8)	4
Leading to death	1 (33)	1	0 (0)	0	0 (0)	0	1 (25)	0	2 (25)	2	4 (17)	4

n (%), number (percentage) of patients. HALPC, human allogeneic liver-derived progenitor cells; NoE, number of events.

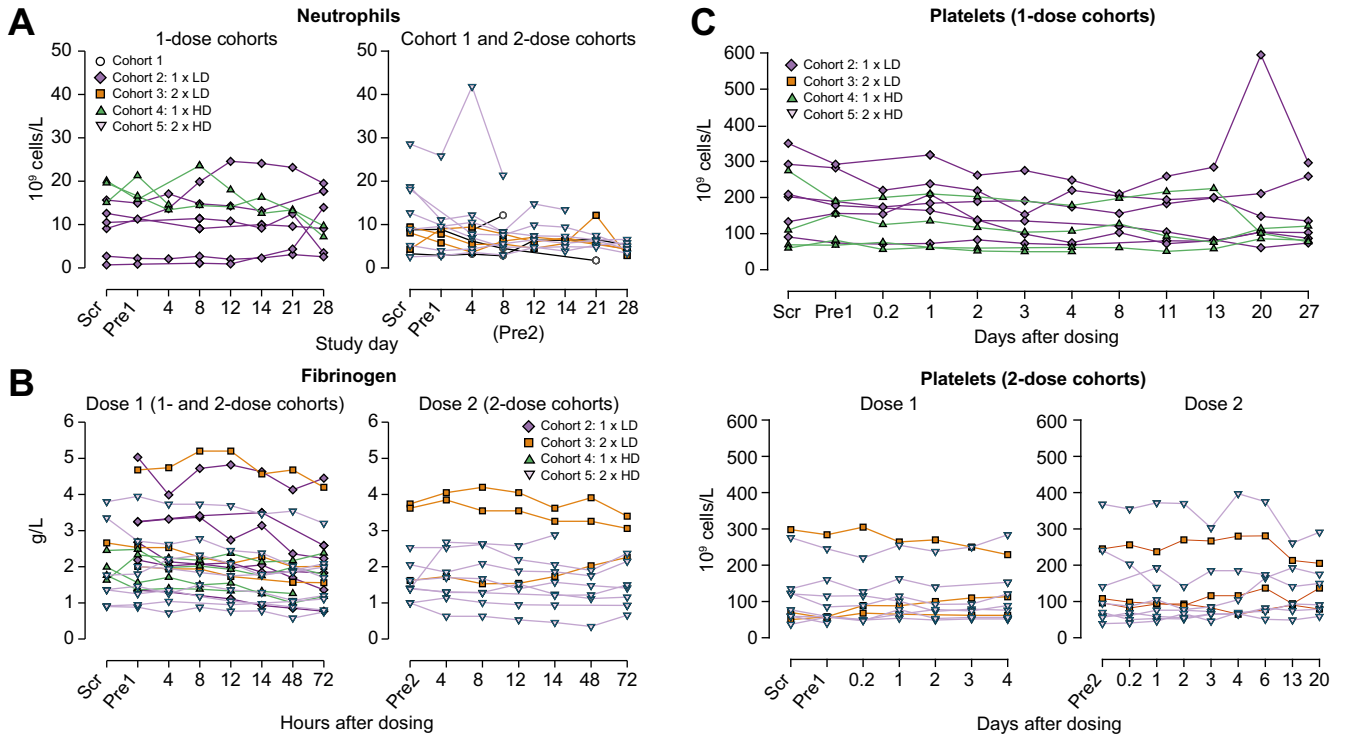

Fig. 2. Haematology measurements in the Safety Analysis set during the treatment and surveillance periods of the study. (A) Neutrophil counts in Cohorts 2 and 4 (left graph) and in Cohorts 1, 3 and 5 (right graph). (B) Fibrinogen values after dosing in Cohorts 2 and 4 (left graph) and in Cohorts 3 and 5 (right graph). (C) Platelet counts in Cohorts 2 and 4 (upper graph) and in Cohorts 3 and 5 within 4 days after the first dose (lower left graph), and within 20 days after the second dose (lower right graph). HD, higher dose of 1.2×10^6 cells/kg BW; LD. lower dose of $0.6–0.8\times10^6$ cells/kg BW; Pre1, pre-dose 1 (Day 1); Pre2, pre-dose 2 (Day 8); Scr, screening.

coagulopathy (clotting disorder) and severe persistent epistaxis after the single infusion. Consequently, the doses were modified downward (to 0.6 or 1.2×10^6 cells/kg BW) and normalised to BW for the remaining 21 patients (Fig. 1B). The allocation to treatment cohorts was also modified in light of that and because of a change in the method to estimate the number of cells administered, such that the first 3 patients were assigned to Cohort 1, and the other 21 patients were assigned to Cohorts 2–5. Given that the Per-Protocol set was smaller (N = 18) than the Safety Analysis set (SAS; N = 24) and that protocol deviations were generally considered as not clinically important, the SAS was also considered for the analysis of survival and efficacy parameters.

Safety of HALPC therapy

In total, 121 AEs were reported by 21 patients of the 24 patients in the SAS during the active study period (Day 1–28; Table 2). The AEs were generally typical of the clinical course of ACLF and AD, with the most frequent AEs being classified as gastrointestinal disorders (36 AEs reported by 13 patients) and infections and infestations (14 AEs reported by 10 patients).

No serious or severe AE was related to HALPC therapy or study procedures with the regimens that included up to 2 doses of 1.2×10^6 cells/kg BW (Cohorts 2–5; Table 2). The 4 serious and severe AEs related to HALPC therapy and study procedures were reported for the 2 SUSAR patients in Cohort 1. Three of these AEs constituted the 2 SUSARs. Liver transplantation was the fourth AE and was reported as related to HALPC therapy for the patient who had the severe-bleeding SUSAR. Two other AEs related to HALPC therapy, flushing and non-haemorrhagic vomiting, were non-serious and mild in intensity and were reported for 2 patients in Cohort 2. One AE (flushing) was also related to study procedures and resolved on the same day. The other AE (non-haemorrhagic vomiting) was followed by the Mallory-Weiss syndrome which was not related to HALPC therapy; these sequelae resolved within 3 days. This latter patient also had an episode of bilious vomiting 2 weeks before inclusion in the study.

Twenty-one of the 25 severe AEs, reported for 9 patients, were unrelated to HALPC therapy or study procedures, but were typical of the clinical course of ACLF and AD (Table 2). Twelve of the 21 AEs were serious, including 3 liver transplants and 4 that were fatal. The 4 fatalities occurred on Day 11 (decompensated liver cirrhosis; Cohort 1), Day 7 (multiple organ failure; Cohort 4), and Days 10 and 18 (septic shock and decompensated liver cirrhosis, respectively; Cohort 5).

The haematological and biochemical parameters for the majority of the SAS patients were not clinically significantly abnormal at baseline (Day 1) or during the active study period. There was no consistent temporal connection between clinically significant abnormal values and HALPC therapy. For example, clinically significant abnormal neutrophil counts were reported for 2 patients in Cohort 4 before treatment (Fig. 2A). During the active study period, clinically significant abnormal counts were reported again for 1 of those patients in Cohort 4 who later died of multiple organ failure on Day 7, and for 1 patient in Cohort 5 who died of septic shock on Day 10.

There was no clear evidence that HALPC infusion in the dose range $0.6–1.2\times10^6$ cells/kg BW increased the risk of bleeding. For

certain parameters associated with clotting, such as fibrinogen and platelets, more frequent measurements with the amended protocol were performed over the 3-day period(s) after dosing (Fig. 2B and C). Nevertheless, the values for these parameters remained relatively stable, even though most fibrinogen concentrations and platelet counts at baseline and over the 3-day period after dosing were lower than normal (<2 g/l and <150 $\times 10^9$/ml, respectively) for a number of patients (10/21 and 14/21, respectively).

As expected in patients with cirrhosis,[32] mean values for factor VIII appeared elevated, whereas those for factor II and factor VII were low compared with expected normal ranges. The levels of protein C, protein S, and antithrombin III were lower than the normal ranges at screening (not shown), as expected in patients with cirrhosis. However, no clinically significant decreases in coagulation factors were reported within 24 h after HALPC infusion.

Before infusion, D-dimer concentrations were in line with the high concentration generally observed in patients with cirrhosis (>5 mg/l).[33,34] A transient increase, reaching its maximum at 4–8 h after HALPC infusion was observed in most patients. In the 4 h after infusion, fibrin clot formation or fibrinolysis appeared unaffected based on TEG data. Thrombin generation also appeared unaffected as illustrated by endogenous thrombin potential peak values.

No thrombotic event was detected after HALPC infusion and no specific signal indicating a perturbation in the coagulation balance was detected except for 2 patients with pre-existing coagulopathy who had coagulation failure and high INR at screening, which further increased after infusion. One of these patients also had a very low fibrinogen level.

No AEs arising from vital signs or physical examinations (*e.g.* hypotension, hypertension, or findings associated with intestinal, pulmonary, cardiac, neurological systems, and skin) were related to HALPC therapy or cell infusion. Liver parenchyma examination showed abnormalities in the majority of patients at baseline (15/24), and at the end of the active study period at Day 28 (12/16). The portal vein was patent for all patients at baseline and remained as such up to Day 28. Abnormal cardiac Doppler ultrasound values were reported for 10/24 patients at baseline and 5/24 patients on Day 1. No major changes were observed in the 24 h after HALPC infusion.

From Day 28 to Month 3, 3 more patients died from disease complications, 1 patient was hospitalised for AD and 1 patient received a liver transplant (Table 3). No additional laboratory abnormalities were reported.

There was evidence of some immune recognition of HALPC in some patients. Class II anti-HLA antibodies were detected (>1,500 mean fluorescence intensity) after treatment in 2 of the 4 patients who had Class I anti-HLA antibodies at screening. However, for one of these 2 patients, the Class II anti-HLA antibodies were only detected after liver transplantation. In 4 other patients without Class I (or Class II) anti-HLA antibodies at screening, Class I and/or Class II anti-HLA antibodies were detected after treatment. However, 2 of those patients had also received blood transfusions. In 3 of the 6 patients with anti-HLA antibodies post-treatment, no anti-HLA antibodies were detected in subsequent samples.

Clinical and biochemical observations following HALPC therapy

During the first 3 months of the study, 4 patients in the SAS (N = 24) underwent liver transplantation and 7 patients died, including 1 of the liver transplant patients (Fig. 1B). Hence, the overall survival rate was 83% (20/24) at Day 28 and 71% (17/24) at Month 3, and the transplant-free survival rate was 71% (17/24) at Day 28 and 58% (14/24) at Month 3. When considering only the 15 patients with ACLF at Day 1, the overall survival rate was 73% (11/15) at Day 28 and 53% (8/15) at Month 3, and the transplant-free survival rate was 67% (10/15) at Day 28 and 53% (8/15) at Month 3. Overall, the averages of the prognosis scores for the surviving patients in the SAS were lower at Month 3 than at Day 1 (Table 4), and no patient had ACLF at Month 3.

Of the 21 patients included in the amended protocol (*i.e.* Cohorts 2–5), 18 were assigned for further efficacy analysis (Efficacy Analysis set; Fig. 1B); hence, 3 patients in Cohort 2 (recipients of a single infusion of 0.6×10^6 cells/kg BW) were not considered because they underwent liver transplantation within 29 days after HALPC infusion.

The averages of the prognosis scores for these 18 patients improved over the 3-month follow-up, illustrated by the MELD-Na (transplant prioritisation) and Child-Pugh scores (mortality risk). At Day 28, Months 2 and 3, the average MELD-Na scores of 21 (SD = 7.8), 15 (SD = 5.2) and 15 (SD = 5.2), respectively, were lower than at baseline (27; SD = 4.7). At Month 3, the MELD-Na score was lower than the respective baseline for 12/13 (92%) surviving patients, with MELD-Na score ≤15 for 8/13 (61%) patients. At Day 28, Month 2, and Month 3, the average Child-Pugh scores of 8.8 (SD = 1.7), 7.4 (SD = 2.0) and 6.8 (SD = 1.5), respectively, were lower than at baseline (11; SD = 1.5). At Month 3, the Child-Pugh score was lower than the respective baseline for 12/13 (92%) surviving patients, with Child-Pugh score ≤6 for 7/13 (53%) patients.

Overall, markers of systemic inflammation (*i.e.* CRP and leucocyte count) and altered liver function (*i.e.* serum biochemistry values that contributed to the MELD-Na and Child-Pugh scores) decreased gradually for the surviving patients over the 3-month follow up, most notably at Months 2 and 3 (Fig. 3A). The average

Table 3. Adverse events of special interest (AESIs) occurring between Day 29 and Month 3 (Safety Analysis set).

	AESIs occurring between Day 29 and Month 3											
	Cohort 1 (N = 3)		Cohort 2 (N = 6)		Cohort 3 (N = 3)		Cohort 4 (N = 4)		Cohort 5 (N = 8)		Total (N = 24)	
	n (%)	NoE	n (%)	NoE	n (%)	NoE	n (%)	NoE	n (%)	NoE	n (%)	NoE
Any AESI	0 (0)	0	3 (50)	3	0 (0)	0	0 (0)	0	2 (25)	2	5 (25)	5
Leading to death	0 (0)	0	2 (33)	2	0 (0)	0	0 (0)	0	1 (12)	1	3 (12)	3
Liver transplant	0 (0)	0	1 (17)	1	0 (0)	0	0 (0)	0	0 (0)	0	1 (8)	1
Hospitalisation	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)	0	1 (12)	1	1 (8)	1

n (%), number (percentage) of patients. NoE, number of events.

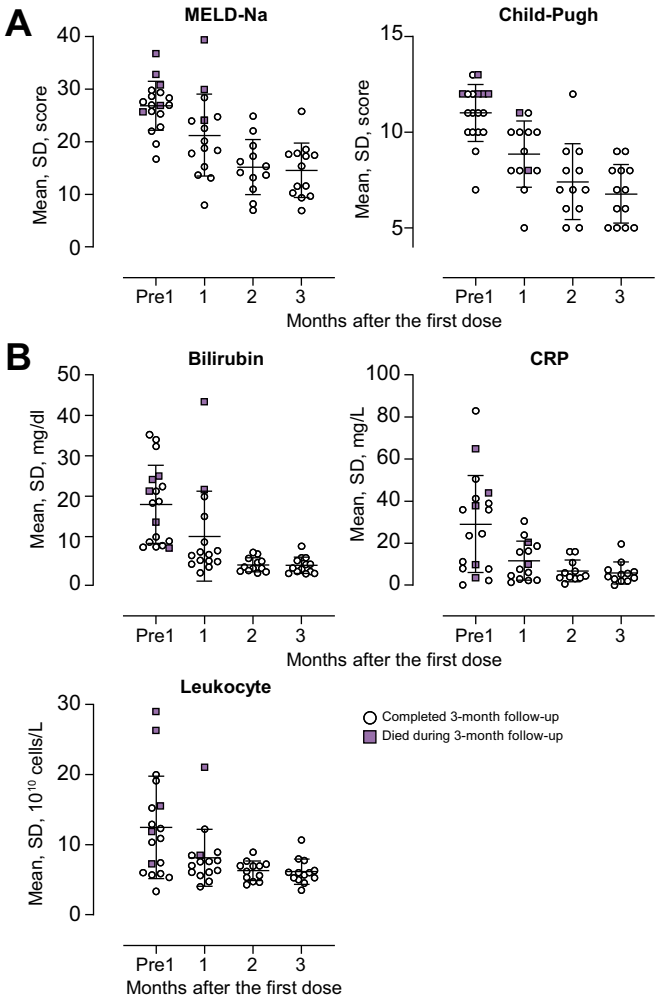

Fig. 3. Prognosis scores and representative serum biochemistry and haematology parameters in the Efficacy Analysis set at baseline, Day 28 (Month 1), Month 2, and Month 3. (A) Means, standard deviations (SDs), and individual patient values for model for end-stage liver disease-sodium (MELD-Na; left graph) and Child-Pugh scores (right graph). (B) Means, SDs, and individual patient values for bilirubin (left graph), C-reactive protein (CRP; middle graph) and leucocytes (right graph). Baseline was pre-dose 1 (Pre1) on Day 1. Values for patients who died during the follow-up are indicated by square orange symbols.

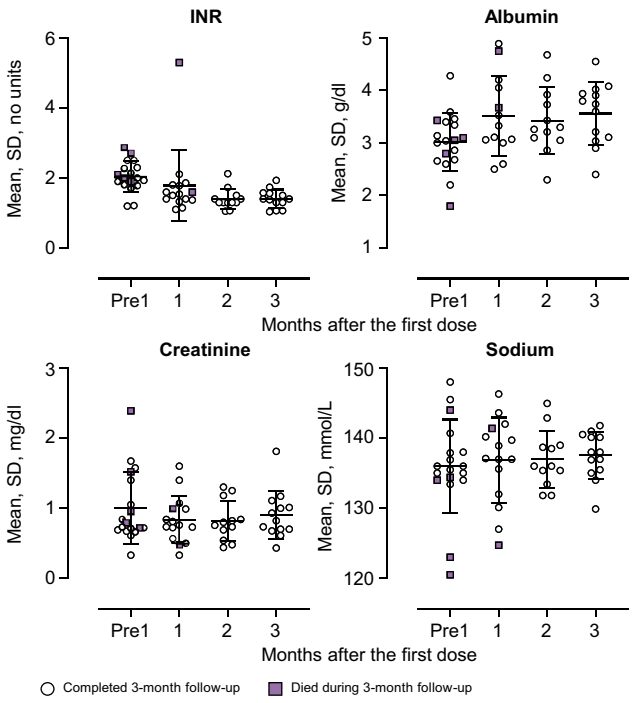

Fig. 4. Additional haematology and serum biochemistry parameters in the Efficacy Analysis set at baseline, Day 28 (Month 1), Month 2, and Month 3. (A) Means, standard deviations (SDs) and individual patient values for international normalised ratio (INR; left graph) and albumin (right graph). (B) Means, SDs and individual patient values for creatinine (left graph) and sodium (right graph). Baseline was pre-dose 1 (Pre1) on Day 1. Values for patients who died during the follow-up are indicated by square orange symbols.

Table 4. Prognosis scores in the Safety Analysis set at baseline, Day 28, and Month 3.

	Day 1 (Baseline)		Day 28		Month 3	
Score*	n	Mean (SD)	n	Mean (SD)	n	Mean (SD)
CLIF-OF	24	9.2 (1.4)	17	7.5 (1.7)	13	6.2 (0.4)
CLIF-C ACLF	15	49.0 (6.9)	4	49.6 (5.7)	0	–
CLIF-C AD	9	51.8 (8.3)	15	49.1 (8.5)	17	44.9 (6.5)
MELD-Na	24	27.2 (4.2)	19	20.9 (8.0)	17	13.1 (5.2)
Child-Pugh	24	10.8 (1.5)	16	8.9 (2.1)	16	6.4 (1.6)

ACLF, acute-on-chronic liver failure; AD, acute decompensation of liver cirrhosis; CLIF, chronic liver failure; MELD-Na, model for end-stage liver disease-sodium; SD, standard deviation.
* Scores were calculated when data were available and allowed the score calculation. Scores were similar when data from patients who underwent liver transplantation were excluded.

bilirubin, CRP, and leucocyte values and their variability (SD) at Months 2 and 3 were lower than at baseline (Fig. 3B). Hence, bilirubin values declined to below 10 mg/dl for all surviving patients at Month 3, including those with CRP values ≥20 mg/l at baseline. Furthermore, the averages and SDs of the INRs followed a similar trend to that of bilirubin values (Fig. 4). By contrast, the average albumin score of 3.6 g/dl (SD = 0.6) at Month 3 was marginally higher than at baseline (3.0 g/dl; SD = 0.6) and was at the low end of the normal reference range (3.5–5.4 g/dl; Fig. 4). Creatinine and sodium levels appeared to be generally stable over the active study period suggesting that any kidney conditions did not worsen as could have occurred (Fig. 4).

Discussion

ACLF is associated with single or multiple organ failure and a high risk of mortality, whereas patients with AD may present at maximum 1 non-renal organ failure and have lower mortality.[1–3] Although a liver transplant represents the only definitive therapeutic option, few ACLF patients receive one owing to advanced age, active alcoholism, uncontrolled infections, and multiple organ failure, leaving an unmet medical need. Spontaneous recovery may occur in a subset of patients under supportive care. HALPC therapy is a medicinal product with a potential to treat ACLF and improve this rate of recovery. Hence, we conducted an open-label phase II clinical study evaluating the safety and preliminary efficacy of HALPC infusions in patients with ACLF or AD. The study population of 24 patients was small but typical of the pathology[1]; and the analysis was descriptive with no formal

hypothesis testing (*i.e.* it was a feasibility study without a control group).

Initially, the dose of HALPC (~5×10^6 cells/kg BW) was similar to that administered with other candidate MSC treatments in other clinical studies, and also shown to be safe in a previous trial of HALPC therapy in children with inborn errors of metabolism.[27,35] However, as a consequence of severe adverse bleeding events attributed to treatment in 2 of the first 3 patients (as explained below), the study was amended to reduce HALPC doses to 0.6 or 1.2×10^6 cells/kg BW, administered either as 1 or 2 infusions 7 days apart. For the subsequent 21 patients, no SAEs were related to treatment, and other AEs were in line with those expected in patients with ACLF and AD, suggesting that the safety profiles of the amended HALPC regimens were acceptable.

The 2 severe bleeding events observed with the high dose regimens may have arisen because the cells may have triggered the consumption of procoagulant factors from an already depleted reservoir of those factors that is a typical feature of cirrhosis.[32] HALPC, as with other MSC or pancreatic islets, and via their expression of tissue factor which can activate the extrinsic coagulation cascade, have known procoagulant activity.[34–36] Nevertheless, MSC have been safely administered by the i.v. route without anticoagulation medication, even if triggering activation of the clotting system.[34,37,38] In this study, some coagulation activity may have been manifested by the transient elevation of serum D-dimer concentration, which is typical of i.v. MSC and other cell-based treatments.[33,34] With respect to the coagulation factor profile, it was consistent with the known profile for patients with cirrhosis[39]: slightly elevated levels of factor VIII, and low levels of factor VII, factor II, protein C, protein S, and antithrombin III. No clinically relevant drop in coagulation factors was recorded 24 h after HALPC infusion. No thrombotic events were observed and thrombin generation, which is generally preserved in patients with AD/ACLF,[40,41] was unaffected after HALPC infusion. There was no specific signal indicating a perturbation in the coagulation balance (detected with the conventional coagulation tests and global tests of clot formation [TEG and TGT]) following i.v. infusions of HALPC at doses of 0.6 or 1.2×10^6 cells/kg BW.

During the first 3 months of the study, 4 patients received liver transplants and 7 patients died (including a transplant recipient). The 3 transplant recipients in Cohort 2 had the operation before Day 28. Hence for the 24 treated patients – all of whom had bilirubin levels above 5 mg/dl at screening – the Day-28 and Month-3 survival rates were 83% and 71%, respectively, and the transplant-free survival rates were 71% and 58%, respectively. In the 15 ACLF patients, the survival rates were 73% and 53%, respectively.

The averages of the prognosis scores for the surviving patients were generally lower (improved) at Month 3 than at baseline, and no patient at Month 3 had ACLF. In those 18 patients, who received $0.6–1.2\times10^6$ cells/kg BW and were not recipients of liver transplants and bilirubin, CRP, and leucocyte values (markers of potential systemic inflammation) were generally lower at Month 3 than at baseline. Creatinine and sodium levels appeared to be generally stable over the 3-month period suggesting that any kidney conditions did not worsen. The suggestion of a progressive increase in albumin over time may have also indicated functional recovery.

In conclusion, this clinical study suggests that the treatment of patients with ACLF or AD with up to 2 doses of 1.2×10^6 HALPC/kg BW is safe. This supports the next phase of clinical development of HALPC therapy, which is a proof-of-concept study in a larger cohort of patients with Grade 1 or Grade 2 ACLF to confirm safety and to demonstrate preliminary evidence of efficacy.

Abbreviations

ACLF, acute-on-chronic liver failure; AD, acute decompensation of liver cirrhosis; AE, adverse event; AESI, AE of special interest; ATMP, advanced therapy medicinal product; BW, body weight; CRP, C-reactive protein; EASL-CLIF, European Association for the Study of Chronic Liver Failure; HALPC, human allogeneic liver-derived progenitor cells; INR, international normalised ratio; i.v., intravenous; MELD, model for end-stage liver disease; MSC, mesenchymal stem cells; SAE, serious AE; SAS, safety analysis set; SUSAR, suspected unexpected serious adverse reaction; TEG, thromboelastography; TGT, thrombin generation test.

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Conflicts of interest

NC-C, IS, NG, YV, SM, and VB are employees of Promethera Biosciences. MN is a founder, patent holder, and consultant of Promethera Biosciences and employee of UCLouvain. ES is a founder, patent holder, consultant and board member of Promethera Biosciences and employee of UCLouvain. TG is an advisory board member for Promethera Biosciences and for Goliver Therapeutics, and is a recipient of a grant from Gilead Sciences Inc. FN, P-FL, LL, LH, VV, DL, and AA report no conflicts of interest.

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

Authors' contributions

Supervised the design and conduct of the trial: ES, NC-C, VB, FN, TG.

Participated in the acquisition, analysis, and interpretation of the data: ES, NC-C, VB, FN.

Drafting the manuscript or revising it critically for important intellectual content: all authors.

Data availability statement

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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Supplementary data

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References

Author names in bold designate shared co-first authorship

[1] Moreau R, Jalan R, Gines P, Pavesi M, Angeli P, Cordoba J, et al. Acute-on-chronic liver failure is a distinct syndrome that develops in patients with

acute decompensation of cirrhosis. Gastroenterology 2013;144. 1426–37, 37 e1–9.
[2] Arroyo V, Moreau R, Kamath PS, Jalan R, Gines P, Nevens F, et al. Acute-on-chronic liver failure in cirrhosis. Nat Rev Dis Prim 2016;2:16041.
[3] Arroyo V, Moreau R, Jalan R. Acute-on-chronic liver failure. N Engl J Med 2020;382:2137–2145.
[4] Trebicka J, Amoros A, Pitarch C, Titos E, Alcaraz-Quiles J, Schierwagen R, et al. Addressing profiles of systemic inflammation across the different clinical phenotypes of acutely decompensated cirrhosis. Front Immunol 2019;10:476.
[5] **Katoonizadeh A**, **Laleman W**, Verslype C, Wilmer A, Maleux G, Roskams T, et al. Early features of acute-on-chronic alcoholic liver failure: a prospective cohort study. Gut 2010;59:1561–1569.
[6] Korf H, du Plessis J, van Pelt J, De Groote S, Cassiman D, Verbeke L, et al. Inhibition of glutamine synthetase in monocytes from patients with acute-on-chronic liver failure resuscitates their antibacterial and inflammatory capacity. Gut 2019;68:1872–1883.
[7] **Moreau R**, **Claria J**, **Aguilar F**, **Fenaille F**, Lozano JJ, Junot C, et al. Blood metabolomics uncovers inflammation-associated mitochondrial dysfunction as a potential mechanism underlying ACLF. J Hepatol 2020;72:688–701.
[8] Mahmud N, Kaplan DE, Taddei TH, Goldberg DS. Incidence and mortality of acute-on-chronic liver failure using two definitions in patients with compensated cirrhosis. Hepatology 2019;69:2150–2163.
[9] Shi Y, Yang Y, Hu Y, Wu W, Yang Q, Zheng M, et al. Acute-on-chronic liver failure precipitated by hepatic injury is distinct from that precipitated by extrahepatic insults. Hepatology 2015;62:232–242.
[10] Li H, Chen LY, Zhang NN, Li ST, Zeng B, Pavesi M, et al. Characteristics, Diagnosis and prognosis of acute-on-chronic liver failure in cirrhosis associated to hepatitis B. Sci Rep 2016;6:25487.
[11] European Association for the Study of the Liver. EASL Clinical Practice Guidelines for the management of patients with decompensated cirrhosis. J Hepatol 2018;69:406–460.
[12] Banares R, Nevens F, Larsen FS, Jalan R, Albillos A, Dollinger M, et al. Extracorporeal albumin dialysis with the molecular adsorbent recirculating system in acute-on-chronic liver failure: the RELIEF trial. Hepatology 2013;57:1153–1162.
[13] Gerth HU, Pohlen M, Tholking G, Pavenstadt H, Brand M, Husing-Kabar A, et al. Molecular adsorbent recirculating system can reduce short-term mortality among patients with acute-on-chronic liver failure – a retrospective analysis. Crit Care Med 2017;45:1616–1624.
[14] **Sanyal AJ**, **Boyer T**, Garcia-Tsao G, Regenstein F, Rossaro L, Appenrodt B, et al. A randomized, prospective, double-blind, placebo-controlled trial of terlipressin for type 1 hepatorenal syndrome. Gastroenterology 2008;134:1360–1368.
[15] Chan AC, Fan ST. Criteria for liver transplantation in ACLF and outcome. Hepatol Int 2015;9:355–359.
[16] Allen AM, Kim WR, Moriarty JP, Shah ND, Larson JJ, Kamath PS. Time trends in the health care burden and mortality of acute on chronic liver failure in the United States. Hepatology 2016;64:2165–2172.
[17] Le Blanc K, Frassoni F, Ball L, Locatelli F, Roelofs H, Lewis I, et al. Mesenchymal stem cells for treatment of steroid-resistant, severe, acute graft-versus-host disease: a phase II study. Lancet 2008;371:1579–1586.
[18] Caplan AI. Mesenchymal stem cells: time to change the name! Stem Cell Transl Med 2017;6:1445–1451.
[19] **Shi M**, **Zhang Z**, **Xu R**, Lin H, Fu J, Zou Z, et al. Human mesenchymal stem cell transfusion is safe and improves liver function in acute-on-chronic liver failure patients. Stem Cell Transl Med 2012;1:725–731.
[20] **Lin BL**, **Chen JF**, Qiu WH, Wang KW, Xie DY, Chen XY, et al. Allogeneic bone marrow-derived mesenchymal stromal cells for hepatitis B virus-related acute-on-chronic liver failure: a randomized controlled trial. Hepatology 2017;66:209–219.
[21] Najimi M, Khuu DN, Lysy PA, Jazouli N, Abarca J, Sempoux C, et al. Adult-derived human liver mesenchymal-like cells as a potential progenitor reservoir of hepatocytes? Cell Transpl 2007;16:717–728.
[22] Sokal EM, Lombard CA, Roelants V, Najimi M, Varma S, Sargiacomo C, et al. Biodistribution of liver-derived mesenchymal stem cells after peripheral injection in a hemophilia a patient. Transplantation 2017;101:1845–1851.
[23] **Najar M**, **Crompot E**, Raicevic G, Sokal EM, Najimi M, Lagneaux L. Cytokinome of adult-derived human liver stem/progenitor cells: immunological and inflammatory features. Hepatobil Surg Nutr 2018;7:331–344.
[24] Lombard CA, Sana G, LeMaoult J, Najar M, Ravau J, Andre F, et al. Human hepatocytes and differentiated adult-derived human liver stem/progenitor cells display in vitro immunosuppressive properties mediated, at least in part, through the nonclassical HLA Class I molecule HLA-G. J Immunol Res 2019;2019. 8250584.
[25] El-Kehdy H, Sargiacomo C, Fayyad-Kazan M, Fayyad-Kazan H, Lombard C, Lagneaux L, et al. Immunoprofiling of adult-derived human liver stem/progenitor cells: impact of hepatogenic differentiation and inflammation. Stem Cell Int 2017;2017. 2679518.
[26] **Najimi M**, **Berardis S**, **El-Kehdy H**, **Rosseels V**, Evraerts J, Lombard C, et al. Human liver mesenchymal stem/progenitor cells inhibit hepatic stellate cell activation: in vitro and in vivo evaluation. Stem Cell Res Ther 2017;8:131.
[27] **Smets F**, **Dobbelaere D**, McKiernan P, Dionisi-Vici C, Broue P, Jacquemin E, et al. Phase I/II trial of liver-derived mesenchymal stem cells in pediatric liver-based metabolic disorders: a prospective, open label, multicenter, partially randomized, safety study of one cycle of Heterologous Human Adult Liver-derived Progenitor Cells (HepaStem) in urea cycle disorders and Crigler-Najjar syndrome patients. Transplantation 2019;103:1903–1915.
[28] Scheers I, Maerckx C, Khuu DN, Marcelle S, Decottignies A, Najimi M, et al. Adult-derived human liver progenitor cells in long-term culture maintain appropriate gatekeeper mechanisms against transformation. Cell Transpl 2012;21:2241–2255.
[29] International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH). E6 (R2) Good Clinical Practice (GCP) Guideline. 2016.
[30] World Medical Association (WMA). Declaration of Helsinki. 2013.
[31] European Medicines Agency (EMA-CHMP). Guideline on Safety and Efficacy Follow-up and Risk Management of Advanced Therapy Medicinal Products: EMEA/149995/2008. London: EMA; 2008.
[32] Kujovich JL. Coagulopathy in liver disease: a balancing act. Hematol Am Soc Hematol Educ Program 2015;2015:243–249.
[33] Perlee D, van Vught LA, Scicluna BP, Maag A, Lutter R, Kemper EM, et al. Intravenous infusion of human adipose mesenchymal stem cells modifies the host response to lipopolysaccharide in humans: a randomized, single-blind, parallel group, placebo controlled trial. Stem Cells 2018;36:1778–1788.
[34] Moll G, Ignatowicz L, Catar R, Luecht C, Sadeghi B, Hamad O, et al. Different procoagulant activity of therapeutic mesenchymal stromal cells derived from bone marrow and placental decidua. Stem Cell Dev 2015;24:2269–2279.
[35] Coppin LCF, Smets F, Ambroise J, Sokal EEM, Stephenne X. Infusion-related thrombogenesis by liver-derived mesenchymal stem cells controlled by anticoagulant drugs in 11 patients with liver-based metabolic disorders. Stem Cell Res Ther 2020;11:51.
[36] Stephenne X, Nicastro E, Eeckhoudt S, Hermans C, Nyabi O, Lombard C, et al. Bivalirudin in combination with heparin to control mesenchymal cell procoagulant activity. PloS One 2012;7:e42819.
[37] Moll G, Ankrum JA, Kamhieh-Milz J, Bieback K, Ringden O, Volk HD, et al. Intravascular mesenchymal stromal/stem cell therapy product diversification: time for new clinical guidelines. Trend Mol Med 2019;25:149–163.
[38] Moll G, Alm JJ, Davies LC, von Bahr L, Heldring N, Stenbeck-Funke L, et al. Do cryopreserved mesenchymal stromal cells display impaired immunomodulatory and therapeutic properties? Stem Cells 2014;32:2430–2442.
[39] Muciño-Bermejo J, Carrillo-Esper R, Uribe M, Mendez-Sanchez N. Coagulation abnormalities in the cirrhotic patient. Ann Hepatol 2013;12:713–724.
[40] Tripodi A, Salerno F, Chantarangkul V, Clerici M, Cazzaniga M, Primignani M, et al. Evidence of normal thrombin generation in cirrhosis despite abnormal conventional coagulation tests. Hepatology 2005;41:553–558.
[41] **Fisher C**, **Patel VC**, Stoy SH, Singanayagam A, Adelmeijer J, Wendon J, et al. Balanced haemostasis with both hypo- and hyper-coagulable features in critically ill patients with acute-on-chronic-liver failure. J Crit Care 2018;43:54–60.