SAFETY SUMMARY

Study design:

The study was an interventional, multicenter, open, non-controlled study of different dose regimens of HepaStem[1] given in subsequent cohorts in participants hospitalized for ACLF/AD and/or who developed ACLF during hospitalization. It was initially planned to enroll a total of 12 participants in 2 subsequent cohorts exploring increasing dose levels (Cohort 1: $250x10^6$ cells in 50 mL/infusion, 4 infusions; and Cohort 2: $500x10^6$ cells in 100 mL/infusion, 4 infusions). Following the occurrence of 2 serious adverse events (SAEs) of bleeding in the last 2 of the first 3 participants enrolled in Cohort 1, the study was halted and the study protocol was amended based on the recommendations from the study safety monitoring committee (SMC): 1/ recruited cirrhotic participants were to present less severe stage of ACLF or AD at risk of developing ACLF, 2/ the infused dose was divided by approximately 10 and adapted to the participant's body weight (BW), 3/ participant monitoring was intensified prior to and after HepaStem infusion. The dose escalation scheme was also revised.

In the amended study protocol (approved by independent ethics committees [IEC]; refer to Section 5.1), the first 3 participants already dosed constituted Cohort 1a, and the next 3 participants, who constituted Cohort 1b, received an intended dose which was divided by approximately 10, adjusted to the participant's BW (intended dose of $0.25x10^6$ cells/kg BW/infusion) and administered in a single infusion. In order to explore different schemes of administration, participants enrolled in Cohort 2a received a single infusion of twice the dose given in Cohort 1b (intended dose of $0.5x10^6$ cells/kg BW/infusion), and participants enrolled in Cohort 2b received 2 infusions of an intended dose of $0.5x10^6$ cells/kg BW/infusion, one week apart.

The study protocol was then further amended to allow the recruitment of patients at a more severe stage of the disease (Cohort 2b: participants with international normalized ratio [INR] > 2) and increase the dose of HepaStem (Cohorts 2c and 2d received an intended dose of $1.0x10^6$ cells/kg BW during one infusion or 2 repeated infusions, respectively).

As part of process manufacturing improvement, Promethera Therapeutics (named Promethera Biosciences until early 2021) switched the quality control process of determining the number of cells in the clinical batch from manual methods (used during the preparation of the batches before freezing) towards a validated automated method (used after thawing and reconstitution). The new quality control process has been applied on the clinical batches used for HEP101. Differences were observed between the intended cell concentration assessed before freezing (as per manually calculated total cell count [TCC]/mL) and the determined cell concentration after thawing and reconstitution (as per automated calculated TCC/mL). Based on the TCC defined for each batch used during the study, actual doses were evaluated and the cohorts were redefined. The re-defined cohorts are summarized below:

- Cohort 1, first participants enrolled ($4.2x10^6$ cells/kg BW/infusion, twice or $5.3x10^6$ cells/kg BW/infusion, once): N=3, initial Cohort 1a.
- Cohort 2 ($0.6\text{-}0.8x10^6$ cells/kg BW/infusion): N=6, all participants from initial Cohort 1b and 2a.
- Cohort 3 ($0.6x10^6$ cells/kg BW/infusion, twice): N=3, 3 participants from initial Cohort 2b.
- Cohort 4 ($1.2x10^6$ cells/kg BW/infusion): N=4, all 3 participants from initial Cohort 2c plus one participant from initial Cohort 2b.
- Cohort 5 ($1.2x10^6$ cells/kg BW/infusion, twice): N=8, all 5 participants from initial Cohort 2d plus 3 participants from initial Cohort 2b.

The study was divided in 3 periods:

- The **screening period** lasted up to 7 days following informed consent signature and allowed assessing participant's eligibility.
- The **active study period** lasted 28 days (± 2 days) and was divided in 2 sub-periods: the treatment period during which HepaStem was infused and the surveillance period.

Participants were hospitalized during the screening and the treatment periods.

- The **long-term safety follow-up period** allowed the safety monitoring of the participants up to one-year post first HepaStem infusion.

After completion of the study, all participants who received at least one infusion of HepaStem were invited to participate in the long-term follow-up PROLONGSTEM study for 5 additional years (EudraCT: 2017-003989-27).

[1] In the protocols, the investigational medicinal product was named "Heterologous Human Adult Liver-Derived Progenitor Cells (HHALPC)". The name of the cells has been updated after the last version of the protocol to Human Allogeneic Liver-Derived Progenitor Cells (HALPC). The new name is used throughout the CSR.

A SMC was appointed to periodically review and evaluate the accumulated data, to review severe coagulation events related to HepaStem, to provide recommendations to Promethera Therapeutics regarding the continuation, modification or termination of the trial and to assess the overall performance of the study.

Diagnosis and main criteria for inclusion:

Patients included in the study were adults suffering from cirrhosis as diagnosed by liver histology or clinical and imaging examination. Other eligibility criteria were updated between enrollment in Cohorts 1a (study protocol versions [v]1.0 dated 25-Mar-2016 and 2.0 dated 13-Dec-2016) and 1b (study protocol v3.2 dated 11-May-2017), as well as before the enrollment of the last participants in Cohort 2b and participants in Cohorts 2c and 2d (study protocol v5.0 dated 14-Dec-2018 or 6.0 dated 14-Dec-2018):

Patients with cirrhosis as diagnosed by liver histology or clinical and imaging examination are:

- Participants in Cohort 1a:
  ACLF grade 1 (liver failure plus cerebral and/or kidney dysfunction OR renal failure plus cerebral dysfunction OR cerebral failure plus kidney dysfunction OR coagulation failure plus cerebral and/or kidney dysfunction) or ACLF grade 2 (any combination of 2 organ failures including: liver failure, renal failure, cerebral failure, coagulation failure)
- Participants in Cohorts 1b, 2a and 2b (participants with INR < 2):
  Patients with AD, serum total bilirubin ≥ 6 mg/dL (≥ 100 µmol/L), 1.2 ≤ INR < 2
- Participants in Cohorts 2b, 2c and 2d (participants with INR > 2):
  Patients with AD, serum total bilirubin ≥ 6 mg/dL (≥ 100 µmol/L)

Main exclusion criteria included absence of portal vein flow, history of prothrombotic disease or thrombotic events, circulatory failure treated with vasoconstrictors or inotropes, respiratory disorders with pulse oximetry < 93% (and related clinical signs), renal failure (secondary to chronic kidney disease), malignancies, Model for end-stage liver disease severity (MELD) score > 35 (except for participants enrolled under the study protocol v3.2 or v4.0 where MELD > 30). According to the amended study protocol v3.2 (Cohorts 2 and 3), participants with coagulation disorders defined as INR ≥ 2, fibrinogen < 100 mg/dL, platelets < 50'000/mm^3, and participants who underwent major invasive procedure within 4 weeks before infusion were also excluded from the study. From the study protocol v5.0 onwards (Cohort 3, 4 and 5), the definition of coagulation disorders was updated to fibrinogen < 80 mg/dL and platelets < 40'000/mm^3.

Safety objectives:

Primary: The primary objective was to assess the safety of different regimens of HepaStem in cirrhotic patients presenting with acute-on-chronic liver failure (ACLF) or with acute decompensation (AD) at risk of developing ACLF up to Day 28 of the active study period.

Secondary: Long-term safety. To assess the safety of HepaStem up to Month 3 and Year 1 post first HepaStem infusion.

Safety endpoints:

Primary:

- Adverse events (AEs) reported up to Day 28 of the active study period, assessed for seriousness, severity, relationship to HepaStem and/or HepaStem administration procedure (including clinically significant changes in clinical examinations, vital signs, laboratory tests, abdominal echography and Doppler up to Day 28).

Secondary (Long-term Safety):

- Adverse events of special interest (AESIs) assessed up to Month 3 and Year 1: SAEs with fatal outcome, malignancies, AEs assessed by the investigator as possibly related to HepaStem, liver transplantation and outcome.
- New ACLF episodes.

HepaStem Exposure:

The first participant enrolled in Cohort 1 received one infusion of HepaStem following the initial planned regimen ($250x10^6$ cells/infusion). However, due to low flow rate resulting from the lack of agitation of the cell suspension, no or a limited number of cells was administrated. This participant was later discontinued from the study prior to the second infusion due to an undetectable vein flow on ultrasound (US) Doppler. For the subsequent infusions, cells were re-suspended by regular gentle agitation. The 2 other participants in Cohort 1 received respectively 2 and one HepaStem infusions but prematurely discontinued HepaStem treatment following the occurrence of SAEs of severe bleeding. Following study protocol amendments, the dose was reduced, adapted to BW and the frequency of infusions limited. Thus, participants in Cohorts 2 and 4 received one infusion of HepaStem and Cohorts 3 and 5 received a double infusion. Cohorts 2 and 3 received a low dose ($0.6\text{-}0.8x10^6$ cells/kg BW/infusion) and Cohorts 4 and 5 a high dose ($1.2x10^6$ cells/kg BW/infusion) of HepaStem.

Adverse events

During the administration of HepaStem in the first cohort, 2 serious cases of severe bleedings were observed for 2 of the first 3 infused participants. Each case involves 2 serious adverse drug reactions that have been assessed unexpected and related to the investigational medicinal product (IMP) (SUSARs). These events led to a temporary interruption of the study and the study protocol was amended before resuming the study. During the active study period (meaning up to Day 28), a total of 121 events were reported for 21 of the 24 participants included in the SAF. Twenty-five of them were severe in intensity and 18 were serious (reported for 11 and 10 participants, respectively). Except for one SAE (hepatorenal syndrome, Cohort 2), which was assessed as moderate in intensity, all the other SAEs were severe. Four led to death (2 events of decompensated hepatic cirrhosis, one event of septic shock and one event of multiple organ failure). Additionally, 3 participants underwent liver transplantation (one participant in Cohort 1 and 2 in Cohort 2).

All SAEs but one (renal failure, Cohort 2) were resolved/had recovered by the end of the 28-day active study period. Four SAEs (including the 2 serious bleeding) reported for 2 participants in Cohort 1, led to HepaStem discontinuation. They were all severe and related to HepaStem, study procedures or the studied disease. Two other events were assessed as related to HepaStem (flushing and non-hemorrhagic vomiting, reported for one participant each in Cohort 2). They were non-serious, mild in intensity, not related to the studied disease and only one of them (flushing) was related to study procedure. The most common AEs were gastrointestinal disorders (36 events reported for 13 participants) and infections and infestations (14 events reported for 10 participants), mainly expected in the context of the studied pathology and considered as related to the participants' condition.

Between Day 28 and the end of Month 3, 5 AESIs were reported for 5 participants: 3 events with a fatal outcome (one event of pneumatosis intestinalis and 2 events of chronic hepatic failure, Cohorts 2 and 5), one participant was hospitalized for ACLF (Cohort 5) and one underwent liver transplantation (Cohort 2). The 5 events were serious, severe in intensity, and unrelated to HepaStem or study procedures.

Between Month 3 and Year 1, 2 hospitalization events were reported for 2 participants due to progressive worsening condition. One had a fatal outcome (Cohort 5) and the other was resolved after about one week (Cohort 4). These events were considered serious, severe in intensity, and unrelated to HepaStem or study procedures.

Laboratory tests, vital signs, physical examination and other safety assessments

Overall, the majority of the participants had normal or abnormal non-clinically significant (NCS) biochemical parameters at baseline (Day 1) and remained as such for 28 days after HepaStem infusion. For some participants, some values shifted from normal or abnormal NCS to abnormal clinically significant (CS) at one or more time points during the active study period: hemoglobin (2 participants), white blood cells and hematocrit (one participant), creatinine (4 participants), CRP (one participant), potassium (one participant), urea (2 participants) and blood urea nitrogen ([BUN] one participant).

Following the protocol amendment subsequent to the SUSARs, close monitoring of the coagulation factors and parameters was set-up for the subsequent cohorts. At baseline, mean values for Factor VIII appeared high while Factors II and VII were low compared to the expected normal ranges, which is a common profile in cirrhotic patients. For several participants, Factors V, IX and XI were below the normal ranges and mean values were close to the lower limit. No clinically relevant drop in coagulation factors was recorded within the first 24h following HepaStem infusion.

Overall, activated partial thromboplastin time (aPTT) and INR, as well as levels of fibrinogen and platelet counts remained stable during the first 72h following HepaStem infusion. No major variations were observed in their mean levels up to the end of the active study period. Mean platelet values fluctuated but generally remained within 100 and $250x10^9$/L during the first 72h post infusion. At baseline, D-dimer concentrations were in line with the high concentration generally observed in cirrhotic patients (> 500 µg/L). A transient increase reaching its maximum 4-8h after infusion was observed in most of the cohorts (except for the lowest dose cohort). According to the TEG and TGT results obtained within 4h following HepaStem infusion, infused cells did not seem to interfere with fibrin clot formation nor influence fibrinolysis. This suggests that HepaStem infusion in the dose range of $0.6\text{-}1.2x10^6$ cell/kg BW did not increase the risk of bleeding. No thrombotic event was detected following HepaStem infusion and no specific signal indicating a perturbation in the coagulation balance was detected except for 2 participants with pre-existing coagulopathy who exhibited high INR at baseline which further increased after infusion. One of these participants also had very low fibrinogen levels. No additional laboratory abnormalities were reported between Day 28 and Year 1.

Vital signs and physical examination

Abnormal findings observed in vital signs and physical examination (few episodes of hypotension or hypertension and findings in abdomen, pulmonary, cardiac, neurologic and skin systems), when reported as AEs, were not considered as related to HepaStem.

Liver parenchyma examination showed abnormalities in the majority of participants at baseline (in 16 out of 24 participants) and at the end of the active study period (Day 28, in 12 out of 16 participants). Portal vein was patent for all participants at baseline and remained as such for 28 days after HepaStem infusion. Abnormal findings on cardiac US Doppler were reported for 10 and 5 (out of 24) participants during the screening period and on Day 1 after the first infusion, respectively. No major changes were observed in the 24h following HepaStem infusion.
Conclusion: No safety signal was evident after up to 2 HepaStem infusions at a dose between 0.6 to $1.2x10^6$ cells/kg BW/infusion. Moreover, in the same dose range, HepaStem seemed not to disturb the fragile coagulation balance in cirrhotic participants with ACLF/AD. The pandemic caused by the novel SARS-CoV-2 virus had no impact on the conduct of this trial: no SARS-CoV-2 related AEs were reported in the participants of this study.